Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; THES=ASSIGNEE; PLUR=YES; OP=AND		
L14	(differential adj display) same (APC or (dendritic adj cell))	16	L14
L13	L12 not L7	26	L13
L12	L11 and L6	28	L12
L11	L10 and L5	357	L11
L10	L9 and (APC or (dendritic adj cell))	756	L10
L9	(differential adj (screening or expression)) and (tumor or cancer)	4089	L9
L8	L7 and (differential adj (screening or expression))	2	L8
L7	L6 same (transfected or transformed or (genetically adj modified))	24	L7
L6	L5 same (APC or (dendritic adj cell))	300	L6
L5	(array or multiple) same (antigens)	10443	L5
L4	Giedlin-martin.in.	1	L4
L3	L1 and (array same antigens)	1	L3
L2	L1 and (APC)	3	L2
L1	Williams-Lewis-T$.in.	70	L1

END OF SEARCH HISTORY

Status: Path 1 of [Dialog Information Services via Mode

Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
Status: Signing onto Dialog
 ****
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
Welcome to DIALOG
Status: Connected

Dialog level 02.15.02D

Last logoff: 17jun03 14:28:03
Logon file001 20jun03 12:35:30
*** ANNOUNCEMENT ***
***
--File 581 - The 2003 annual reload of Population Demographics is complete. Please see Help News581 for details.

***
--File 156 - The 2003 annual reload of ToxFile is complete. Please see HELP NEWS156 for details.

***
--File 990 - NewsRoom now contains February 2003 to current records. File 992 - NewsRoom 2003 archive has been newly created and contains records from January 2003. The oldest months's records roll out of File 990 and into File 992 on the first weekend of each month. To search all 2003 records BEGIN 990, 992, or B NEWS2003, a new OneSearch category.
***
--Connect Time joins DialUnits as pricing options on Dialog. See HELP CONNECT for information.
***
--CLAIMS/US Patents (Files 340,341, 942) have been enhanced with both application and grant publication level in a single record. See HELP NEWS 340 for information.
***
--SourceOne patents are now delivered to your email inbox as PDF replacing TIFF delivery. See HELP SOURCE1 for more information.
***
--Important news for public and academic libraries. See HELP LIBRARY for more information.
***
--Important Notice to Freelance Authors--
See HELP FREELANCE for more information
***
NEW FILES RELEASED
***World News Connection (File 985)
***Dialog NewsRoom - 2003 Archive (File 992)
***TRADEMARKSCAN-Czech Republic (File 680)
***TRADEMARKSCAN-Hungary (File 681)
***TRADEMARKSCAN-Poland (File 682)
***
UPDATING RESUMED
***
RELOADED
***Population Demographics -(File 581)
***CLAIMS Citation (Files 220-222)

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REMOVED
***U.S. Patents Fulltext 1980-1989 (File 653)

     >>> Enter BEGIN HOMEBASE for Dialog Announcements <<<
     >>>    of new databases, price changes, etc.      <<<
                    ****
KWIC is set to 50.
HILIGHT set on as '*'
* * * * See HELP NEWS 225 for information on new search prefixes
and display codes

***                                    ***


File   1:ERIC  1966-2003/Jun 17
       (c) format only 2003 The Dialog Corporation

      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
?b 155, 159, 5, 73
       20jun03 12:35:52 User259876 Session D518.1
            $0.31    0.089 DialUnits File1
     $0.31  Estimated cost File1
     $0.08  TELNET
     $0.39  Estimated cost this search
     $0.39  Estimated total session cost   0.089 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2003/Jun W3
        (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession numbers have
changed.  Please see HELP NEWS 155.
  File 159:Cancerlit  1975-2002/Oct
        (c) format only 2002 Dialog Corporation
*File 159: Cancerlit ceases updating with immediate effect.
Please see HELP NEWS.
  File   5:Biosis Previews(R)  1969-2003/Jun W3
        (c) 2003 BIOSIS
  File  73:EMBASE  1974-2003/Jun W3
        (c) 2003 Elsevier Science B.V.
*File  73: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
?s ((antigen (w) presenting (w) cell?) or (dendritic (w) cell?)) (s) (array or multiple
 or polyepitope?)
Processing
Processing
Processing
         1098808  ANTIGEN
          245395  PRESENTING
         8712137  CELL?
           39139  ANTIGEN(W)PRESENTING(W)CELL?
           89065  DENDRITIC
         8712137  CELL?
           45691  DENDRITIC(W)CELL?
           77385  ARRAY
         1088772  MULTIPLE
             115  POLYEPITOPE?
      S1    2837  ((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (W)
                  CELL?)) (S) (ARRAY OR MULTIPLE OR POLYEPITOPE?)
?s s1 (s) (transfected or transformed or (genetically (w) modified))
           2837  S1
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          142085  TRANSFECTED
          191036  TRANSFORMED
          140768  GENETICALLY
          468466  MODIFIED
           19839  GENETICALLY(W)MODIFIED
      S2     158  S1 (S) (TRANSFECTED OR TRANSFORMED OR (GENETICALLY (W)
                  MODIFIED))
?s s2 and (differential (w) (display or screening or expression))
             158  S2
          838831  DIFFERENTIAL
          157054  DISPLAY
          536876  SCREENING
         2040746  EXPRESSION
           38823  DIFFERENTIAL(W)((DISPLAY OR SCREENING) OR EXPRESSION)
      S3       0  S2 AND (DIFFERENTIAL (W) (DISPLAY OR SCREENING OR
                  EXPRESSION))
?s s2 and (tumor or cancer)
             158  S2
         2133852  TUMOR
         2259658  CANCER
      S4      78  S2 AND (TUMOR OR CANCER)
?rd
...examined 50 records  (50)
...completed examining records
      S5      36  RD (unique items)
?s s5 and review
              36  S5
         1432113  REVIEW
      S6       4  S5 AND REVIEW
?t s6/3,k/all
```

6/3,K/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09728020 21525235 PMID: 11668436

Genetically modified dendritic cells in *cancer* therapy: implications for transfusion medicine.

Foley R; Tozer R; Wan Y

Department of Laboratory Medicine, Hamilton Health Sciences Corporation, Hamilton, Ontario, Canada.

Transfusion medicine reviews (United States) Oct 2001, 15 (4) p292-304, ISSN 0887-7963 Journal Code: 8709027

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Genetically modified dendritic cells in *cancer* therapy: implications for transfusion medicine.

...susceptible to a variety of gene transfer protocols, which can be used to enhance biological function in vivo. Transduction of DCs with genes for defined *tumor* antigens results in sustained protein expression and presentation of multiple *tumor* peptides to host T cells. Alternatively, DCs may be transduced with genes for chemokines or immunostimulatory cytokines. Although the combination of ex vivo DC expansion and gene transfer is relatively new, preliminary studies suggest that injection of *genetically* *modified* autologous DCs may be capable of generating anti-*tumor* immune responses in patients with *cancer*. Preclinical animal studies showing potent antigen-specific *tumor* immunity after DC-based vaccination support this hypothesis and provide rationale to further evaluate this approach in patients. Preliminary human studies are now required to...

... administration require the collaborative efforts of basic scientists, immunologists, clinical investigators, and transfusion medicine staff to ensure strict quality control of injected cellular products. This *review*

is intended to provide a [redacted]ief overview of clinical DC-bas[redacted] gene transfer.

; Adenoviridae--genetics--GE; *Cancer* Vaccines; Cell Differentiation; Hematopoietic Stem Cells; Monocytes; Neoplasms--immunology--IM; Transfection
Chemical Name: *Cancer* Vaccines

6/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09015549 20308828 PMID: 10850282
***Review*: dendritic cell immunotherapy for melanoma.**
Hadzantonis M; O'Neill H
Division of Biochemistry & Molecular Biology School of Life Sciences, Australian National University, Canberra ACT, Australia.
Cancer biotherapy & radiopharmaceuticals (UNITED STATES) Feb 1999, 14 (1) p11-22, ISSN 1084-9785 Journal Code: 9605408
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

***Review*: dendritic cell immunotherapy for melanoma.**
...elements. Since current therapy for melanoma is limited and associated with high toxicity and side effects, development of alternative approaches is imperative. The importance of *dendritic* *cells* (DCs) in immunity against tumours is now well established. DC immunotherapy for melanoma is possible but must be considered in terms of effectiveness and clinical...

...HPC) in bone marrow or DC progenitors in peripheral blood. Generation of an effective anti-tumour immune response will be dependent upon the presentation of *multiple* melanoma-specific antigens by both major histocompatibility complex (MHC) class I and class II molecules and stimulation of both tumour-specific cytotoxic T lymphocytes (Tc...

... 1 (Th1) cells. Different techniques for delivery of the priming antigen offer different advantages. DCs can be pulsed with peptide, protein or tumour cell lysate, *transfected* with viral vectors or naked nucleic acid and tumour/DC hybridomas can also be generated. Repeated antigen administration into neighbouring lymph nodes appears to be...
; Adjuvants, Immunologic; Algorithms; Antigen Presentation; Antigens, Neoplasm--immunology--IM; *Cancer* Vaccines--immunology--IM; Combined Modality Therapy; Cytokines--genetics--GE; Cytokines--secretion--SE; Cytokines--therapeutic use--TU; DNA--genetics--GE; DNA--immunology--IM; Gene Therapy; Genetic...
Chemical Name: Adjuvants, Immunologic; Antigens, Neoplasm; *Cancer* Vaccines; Cytokines; Genetic Vectors; HLA Antigens; Immunodominant Epitopes; Vaccines, DNA; RNA; DNA

6/3,K/3 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11899952 EMBASE No: 2003011996
Genetically modified dendritic cells - A new, promising *cancer* treatment strategy?
Wysocki P.J.; Grabarczyk P.; Mackiewicz-Wysocka M.; Kowalczyk D.W.; Mackiewicz A.
P.J. Wysocki, Department of Cancer Immunology, Univ. School of Medical Sciences, GreatPoland Cancer Centre, Poznan Poland
AUTHOR EMAIL: pwysocki@plusnet.pl
Expert Opinion on Biological Therapy (EXPERT OPIN. BIOL. THER.) (United Kingdom) 2002, 2/8 (835-845)
CODEN: EOBTA ISSN: 1471-2598

Expert Opinion

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Cell- & Tissue-based Therapy

Genetically modified dendritic cells – a new, promising cancer treatment strategy?

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Dendritic cells (DCs), the most potent antigen-presenting cells (APCs), were discovered almost 30 years ago. Due to the priming of antigen-specific immune responses mediated by CD4+ and CD8+ lymphocytes, DCs are crucial for the induction of adaptive immunity against cancer. Therefore, vaccination of cancer patients with DCs presenting tumour-associated antigens (TAAs) have been believed to be a promising anticancer strategy. Multiple clinical trials have been carried out in order to evaluate the safety and efficacy of cancer vaccines based on antigen-pulsed DCs. However, pulsing of DCs with particular peptides has several disadvantages: i) short-time duration of antigen–major histocompatability complex (MHC) complexes, ii) a requirement for matching defined peptides with MHC complexes and iii) exclusive presentation of single antigen epitopes. Application of gene transfer technologies in the field of DC-based vaccines made possible the development of novel, anticancer immunisation strategies. In several animal models, DCs modified with genes encoding TAA or immunostimulatory proteins have been shown to be effective in the induction of antitumour immune responses. Based on these encouraging results, a first clinical trial of prostate cancer patients vaccinated with gene modified DCs has recently been initiated. In this article, methods used for genetic modification of DCs and anticancer vaccination strategies based on genetically modified DCs are reviewed.

Keywords: cancer vaccine, cytokine, dendritic cell, genetic modification, tumour-associated antigens

Expert Opin. Biol. Ther. (2002) 2(8):835-845

1. Introduction

Dendritic cells (DCs) were discovered almost 30 years ago. Since then, intensive research has proven that they are professional antigen-presenting cells (APCs) [1]. Due to the fact that DCs are crucial for effective induction of adaptive immune responses, DC-based vaccination has been proposed as a promising strategy for cancer treatment [2,3]. Pulsing of DCs with synthetic peptides (epitopes) derived from known tumour-associated antigens (TAAs), such as MART-1/Melan-A, carcinoembryonic antigen (CEA), tyrosinase, prostate specific membrane antigen (PSMA) or glycoprotein 100 (gp100), was associated with induction of an effective, specific antitumour immune response. Currently, several clinical trials are being carried out in order to evaluate the efficacy of this strategy in different cancer types [4,5]. The early results demonstrate induction of antitumour immune responses in some patients immunised with vaccines consisting of TAA-pulsed DCs. In some cases the immunisation has been associated with partial or complete regression of tumours [6,7]. However, pulsing of DCs with peptides has some limits:

 ISSN 1471-2598

- The duration of antigen presentation by DCs is restricted by the half-life of the peptide–major histocompatability complex (MHC) complex created during the antigen pulsing.
- The use of a defined peptide is limited only to patients who express a specific MHC haplotype [8].

Other methods used for generation of DC MHC–antigen peptide complexes are based on genetic modification of DCs and on protein–antigen transduction of DCs [85,86]. Alternatively, hybrid cells (dendritomas) (DCs fused with tumour cells) have been shown to effectively stimulate antitumour immune responses in both animals and humans [104-108]. In this article, methods of genetic modification of DCs and approaches to cancer therapy utilising gene modified DCs are reviewed.

2. The biology of dendritic cells

The effective presentation of antigens by APCs is crucial for the development of an antitumoural immune response, that is, for the priming of naive, tumour-specific T helper and cytotoxic T lymphocytes (CTLs) [9].

The most potent APCs are DCs [1,11], which represent ~ 0.3% of the entire circulating blood-leukocyte population and reside in the spleen, lymph nodes, circulation and peripheral tissues. Although B cells and macrophages can also present antigens to memory T cells, they are far less efficient than DCs [9]. The MHC product and MHC-peptide complexes are 10 – 100 times higher on DCs than on other APCs [10].

DCs residing in peripheral tissues are immature and act as sentinels. In the immature state they efficiently phagocytose both apoptotic and necrotic cells, microbe and particulate antigens, and through macropinocytosis take up soluble proteins. The immature DCs are characterised by a low expression of surface costimulatory molecules and a low level of cytokine secretion. Maturation of DCs is induced by a number of different stimuli which may act as 'danger signals'. Examples include: lipopolysaccharides, apoptotic bodies, heat-shock proteins, activated T cells expressing CD40 ligand, monocyte-conditioned media or cytokines [12,13]. Upon maturation, DCs downregulate the inflammatory chemokine receptors and upregulate a CCR7 chemokine receptor, which makes them responsive to macrophage inflammatory protein-3β (MIP-3β) and 6Ckine which are expressed within the secondary lymphoid organs [14]. Subsequently, maturing DCs migrate to the T cell zones located in these lymphoid organs. During the maturation process DCs upregulate MHC class I and II complexes, adhesive and costimulatory molecules (CD40, CD54, CD80, CD86) and cytokines (for example, IL-4, IL-6, IL-12, IL-15, IL18, IFN-α). They also reorganise the cytoskeleton, developing extensive cytoplasmic veils (for review, see [10]).

The mature human DCs most notably express the CD83 molecule. However, murine DCs lack such an unique marker of maturation. Upon encounters with T cells (CD40–CD40L [ligand] interaction) DCs further upregulate their adhesive and costimulatory molecules and increase secretion of cytokines, thus effectively providing all three signals required for priming naive T cells:

- Presentation of an antigen in the context of MHC I or II complexes to CD8+ or CD4+ lymphocytes, respectively.
- Interaction between DC costimulatory molecules and the respective receptors located on lymphocytes.
- Paracrine secretion of lymphocyte-activating cytokines.

The most important feature of DCs, commonly known as cross-priming, consists of antigen uptake, processing, presentation and priming of antigen-specific T cells. A single DC is efficient for activation of 100 – 3000 T cells [1]. Besides the priming of antigen-specific T cells (adaptive immunity), DCs have also been shown to activate innate effector cells (natural killer [NK] and natural killer T [NKT] cells), which may exhibit antitumour activity [91,92].

3. Generation of dendritic cells

Dendritic cells can be generated employing two different approaches:

- Isolation of immature DC precursors from peripheral blood [15-17].
- The *in vitro* culture of CD34+ progenitor cells or monocytes in the presence of cytokines [18-20].

The first strategy is time-consuming and inefficient (immature DCs precursors represent ~ 0.5% of peripheral blood mononuclear cells [PBMC]). However, this was the first method utilised in clinical studies of DC vaccination.

The peripheral blood circulating CD34+ haematopoietic progenitor cells and CD14+ adherent monocytes cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 differentiate into immature DCs within 5 – 7 days. They lose monocyte markers (CD14 and CD115) and acquire DC markers such as CD1a [19]. Such immature DCs demonstrate high phagocytic and antigen-processing activity [21]. Maturation of these cells can be induced by culture in monocyte conditioned medium or in the presence of particular agents, such as TNF-α, lipopolysaccharide, IFN-α, IL-1β, CD40L or TRANCE. Mature DCs are thought to be more suitable for immunisation regimens than their immature counterparts, mostly due to their stable phenotype, high immunostimulatory capacity and increased trafficking to secondary lymphoid organs [22].

4. Genetic modification of dendritic cells

Genetic material can be transferred into DCs in the form of DNA or RNA, by either viral or non-viral systems.

Non-viral gene delivery methods comprise naked DNA or RNA injection, electroporation, biolistic devices (gene gun)

and liposomes. DCs have been shown to be susceptible to transfection with plasmid DNA [23]. Van Tendeloo and colleagues analysed the efficacy of plasmid DNA transfer into CD34+ progenitor cell-derived DCs, Langerhans cells and monocyte-derived DCs by electroporation and lipofection [24]. The lipofection was totally unsuccessful but the efficacy of gene transfer by electroporation was 16% of Langerhans cells, 12% of CD34+ derived DCs and < 2% of monocyte-derived DCs. The electroporation did not impair allostimulatory functions of DCs [25]. Most physical methods of gene transfection turned out to be toxic to DCs and to induce loss of phenotype and substantial cell death [25]. The 15% DC transfection rate by electroporation is associated with a rapid decrease in their viability (< 60%) [24].

Despite the low level of plasmid DNA transfection, modified DCs are potent stimulators of immune responses. Monocyte-derived DCs transfected with the MART-1 gene were capable of naive CD8+ T-cell priming [26]. Analogically, DCs can be effectively modified with tumour cell-derived RNA [27,28].

The growing consensus is that viral gene delivery methods are far more efficient in modification of different cells than other, non-viral systems. The viral vectors are very efficient in transduction of target DCs but can also disturb their APC functions. There are three viral mechanisms of immune evasion which may hamper the generation of an immune response by infected DCs:

- induction of cell death, inhibition of maturation or downregulation of the costimulatory molecules
- decrease of antigen presentation capacity, for example, downregulation of MHC complexes or disturbance to the TAP (transporter associated with antigen presentation) loading mechanism [29]
- indirect influence on the DC maturation stage by cytokines

For example, infection with a vaccinia virus induces synthesis of TNF receptors and IL-1Rs (interleukin-1 receptors). It further leads to a reduction of the 'danger signals' induced by the viral infection itself [30]. For the *ex vivo* genetic modification of DCs, several viral gene delivery systems are currently utilised. They comprise vectors based on vaccinia viruses, herpes simplex viruses (HSVs), adenoviruses, lentiviruses, retroviruses and adeno-associated viruses (AAVs).

4.1 Vaccinia virus-based vectors

The vaccinia virus belongs to the orthopox virus family. Viral particles are brick-shaped and can carry up to 25 kb of heterologous double-stranded DNA. As a gene delivery tool, they display a relatively broad tropism and a high level of transgene expression. Vaccinia-based viral vectors are derived from highly attenuated virus strains or strains deficient for late gene expression [31,32]. Vaccinia vectors are not capable of transducing CD34+ progenitor cells but can deliver genes into DCs differentiated from such precursors [33]. Vaccinia vectors effectively transduce DCs at a low multiplicity of infection (MOI), and several studies have shown that up to 60% of DCs can be transduced at an MOI of 2.5 [31,34].

4.2 Herpes simplex virus-based vectors

HSV is a large, linear, double-stranded DNA virus with > 80 identified genes located within a 152 kb genome. The wild type viruses are able to both infect cells lytically and to establish latency in specific cell types such as neurons. A recombinant HSV vector enters a latency state in all cells. The major advantage of HSV vectors is the very large capacity for carrying transgenes of up to 50 kb. It makes possible a modification of DCs with several therapeutic genes utilising a single recombinant HSV vector, for example, genes encoding several TAAs along with genes coding for different immunomodulators such as IL-12 and IL-2 [35,36].

4.3 Adenoviral vectors

Adenoviruses are linear, 30 – 35 kb, double-stranded DNA viruses that usually cause upper respiratory tract and eye infections in human. Almost all recombinant adenoviral vectors are derived from human serotypes 2 or 5 of the group C adenovirus. There are several advantages of adenoviral vectors:

- high titre (10^{12} – 10^{13} viral particles/ml)
- ability to infect a wide variety of cell types, including terminally differentiated or postmitotic cells
- accommodation of up to 8 kb (first generation) or up to 30 kb (third generation) of foreign DNA (expression cassettes)

Deletion of the E1 early gene within an adenoviral vector renders it replication deficient and creates a space for cloning of a transgene. Viral proteins encoded by the early genes (E1 –E5) are involved in the induction of antiadenoviral immune responses (humoral and cellular), which limits the duration of transgene expression and precludes secondary *in vivo* administration [87-90]. However, in the case of the *ex vivo* modified DCs, the antiadenovirus immunity may act as an adjuvant, increasing CTL responses against presented antigens. Indeed, in the study of Wan Y and colleagues, murine DCs modified with an adenoviral vector carrying a TAA effectively induced tumour regression even in the presence of a strong antiadenovirus immune response [37]. Similar results were obtained by Kaplan and colleagues who studied the efficacy of DCs transduced with adenovirus encoding the gp100 antigen in a murine model of malignant melanoma [38]. The second generation adenoviral vectors which lack further early genes, for example E3 and E4, are safer, less immunogenic and more efficacious in animal models [39,40] as well as in humans [41].

The uptake of adenoviral vectors is mediated by the $\alpha_V\beta_5$ integrin which is mostly expressed on immature DCs. A successful transduction of mature DCs requires a very high MOI [42]. However, effective transduction of mature DCs (> 90%) at high a MOI (> 100) is not associated with any perturbation of DC function. In order to improve the efficacy of adenovirus-mediated DC transduction, Tilman and

colleagues have modified the vector's tropism by targeting it with a bispecific antibody to CD40 or β_1 integrins on human DCs. Adenoviral vectors targeted to the CD40 receptor not only effectively transduced DCs (at a relatively low MOI [< 100]) but also induced their maturation, as demonstrated by an increased expression of MHC, CD80, CD83, CD86 and IL-12 molecules. Targeted transduction of DCs was also associated with an enhanced expression of a reporter gene [43]. A relatively high DC transduction efficiency was also achieved by mixing adenovirus with liposomes [44] and by utilisation of adenovirus–polyethylenimine complexes in which plasmid DNA was bound to adenovirus particles by polyethylenimine [33,45].

4.4 Retroviral vectors

Retroviruses are single-stranded RNA oncoretroviruses, which infect dividing cells and integrate their own genetic material with the host genome. Moloney murine leukaemia virus-based retroviral vectors are the most frequently used in clinical trials so far. Retroviruses enter the target cells through PiT receptors, the density of which influences the transduction efficiency [46]. The retroviral vectors are made replication incompetent by replacing their *gag, pol* and *env* genes with a coding cassette that usually consists of a marker and therapeutic gene. Retroviral vectors for genetic modification of DCs have two main advantages:

- integration with the host genome, providing long-term gene expression
- lack of immunogenecity due to the removal of genes encoding viral proteins

The major limitation of retroviral vectors is the fact that they transduce only dividing cells. Modification of DCs differentiated from CD34+ progenitors or isolated from peripheral blood is therefore theoretically impossible. However, some investigators have reported an effective transduction of non-dividing DCs [47-49]. A much more feasible approach seems to involve transduction of dividing CD34+ progenitor cells which will be further used for generation of DCs [50,51].

There are two methods of CD34+ cell transduction by a retroviral vector:

- co-culture of CD34+ cells with packaging cell lines, secreting recombinant retroviruses into the medium
- incubation of CD34+ cells in cell-free viral supernatants

Due to safety concerns the second approach is more likely to be used in clinical trials. However, due to the low viral titre (10^{6-7} colony forming units [CFU]/ml) it requires large volumes of retrovirus-containing supernatants. On the contrary, retroviral vectors pseudotyped with a vesicular stomatitis virus G protein can be produced in higher titres (up to 10^9 CFU/ml), are more stable *in vitro* and enter target cells through PiT-independent pathways. The last feature is important, since there is a lack of functional PiT1 and PiT2 expression on haematopoietic stem cells [52].

The average transduction efficacy of bone marrow- and cord blood-derived DCs is ~10 and 20%, respectively [49]. However, Heemskerk and colleagues achieved a 40 – 60% cell transduction rate [53], and Movassagh and colleagues demonstrated that > 70% of DCs derived from cord blood or mobilised progenitors may be transduced by infection with a gibbon ape leukaemia virus-pseudotyped retroviral vector [54]. Transduction of DCs by retroviral vectors is not associated with alterations of their phenotype or function [49].

4.5 Lentiviral vectors

Unlike retroviral vectors, lentiviral vectors based on HIV or simian immunodeficiency virus (SIV) are able to transduce non-dividing cells, including monocyte-derived DCs [55-59]. Gruber and colleagues have demonstrated that HIV-1 based lentiviral vectors can transduce both immature and mature DCs [55]. At an MOI of 5, 26% of immature and 5% of mature DCs have been transduced. At an MOI of 50, the immature DC transduction rate increased to 50%. Immature DCs modified by lentiviral vectors can still be induced to mature and their viability and phenotype are not significantly altered. Negre and colleagues have demonstrated that SIV-based lentiviral vectors were capable of transducing DCs [56]. Unlike HIV-based, vectors the SIV-derived carriers were more effective in transduction of mature than immature DCs (32 versus 10% at an MOI of 10). Moreover, lentiviral vectors are very effective in genetic modification of stem cells or CD34+ progenitor cells which may be further differentiated into DCs.

Although the lentiviral vectors are extremely effective and safe gene delivery tools, there are still some physiological concerns associated with their potential use for clinical trials.

4.6 Adeno-associated viral vectors

AAV is a member of the parvovirus family which comprises small (4.7 kb), non-pathogenic, single-stranded DNA viruses requiring a helper virus (adenovirus or HSV) for replication. AAV is a human virus and 32% of adult humans possess pre-existing antibodies that prevent transfection *in vitro* by the AAV2 serotype from which most AAV vectors are derived. The recombinant AAV vector is able to accommodate up to 4.5 kb DNA between inverted terminal repeats and can infect a wide variety of cells both *in vitro* and *in vivo* [93,94]. Both human peripheral blood [95,96] and murine bone marrow- and spleen-derived DCs [97] can be transduced with AAV vectors. There are three main advantages of recombinant AAVs (rAAVs) in terms of genetic modification of DCs:

- transduction of both dividing and non-dividing cells allows for modification across a broad range of activation or maturation states
- lack of viral coding sequences minimises the competition between transgene and viral peptides for MHC presentation [98-100]
- capacity for persistent transgene expression [109]

Transduction of DCs with rAAV is not associated with changes in DC phenotype nor with an increased allostimulatory capacity as compared with non-transduced DCs [95].

4.7 Limitations associated with use of viral vectors

Most viral vectors used for modification of DCs introduce sequences encoding viral proteins that may interfere with generating a potent antitumour immune response. Viral antigens may mask or suppress induction of an immune response against less potent tumour antigens in a process called immunodominance [101]. Moreover, activated virus-specific CTLs may recognise and kill DCs simultaneously presenting viral and tumour antigens [102]. Although modification of DCs with viral vectors lacking viral sequences leads entirely to expression of the desired transgenes, viral proteins may be still ingested, processed and presented by DCs, so the potential problem of immunodominance may be unavoidable even with these improved vectors [103].

5. Cancer treatment strategies based on gene modified dendritic cells

Tumours can effectively evade the immune system by several mechanisms not only confined to cancer cells, but also related to impaired function of immune responses in a tumour bearing host. The lack of 'danger signal' during the initial phase of tumour growth precludes the involvement of DCs and the subsequent priming of tumour specific immune responses in secondary lymphoid organs. Naive lymphocytes recognising TAAs present on cancer cells do not receive all signals required for their priming and as a consequence become anergised [12,13]. However, DC-activated tumour-specific lymphocytes can effectively destroy tumour cells after recognition of a target antigen presented in the context of MHC molecules on their surface. Therefore, several DC-based anticancer strategies are being developed in order to induce an effective, antitumour immune response in cancer bearing hosts (Figure 1).

5.1 Dendritic cells modified with genes encoding tumour-associated antigens

Modification of DCs with genes encoding whole tumour antigens avoids limitations associated with pulsing of DCs with defined TAA epitopes. Expression of introduced genes is long lasting and the same gene construct may be inserted into DCs derived from different patients (matching of MHC molecules is not required).

Yang and colleagues have analysed the function of DCs in the processing and presentation of epitopes derived from the gp100 antigen [23]. They have pulsed DCs with three defined gp100-derived peptides or transduced them with a vaccinia virus carrying a whole gp100 gene. CTLs generated by the peptide-pulsed DCs lysed only MHC-matched Epstein–Barr virus (EBV) transformed B cells pulsed with the same peptide. However, CTLs generated by DCs expressing the whole gp100 gene efficiently lysed MHC-matched B cell lines pulsed with any of the analysed peptides. Furthermore, CTLs induced by genetically modified DCs exhibited a strong cytotoxic response against allogenic MHC-matched gp100-positive melanoma cells.

Expression of both MHC class I and II complexes and presentation of endogenous or exogenous antigens in the context of those molecules enables DCs to stimulate both CD8+ and CD4+ T cell responses. In the study of Perez-Diez and colleagues, autologous DCs from melanoma patients or healthy donors transduced with an adenoviral vector carrying the MART-1 gene induced both CD4+ and CD8+ T cells *in vitro*. In response to several rounds of stimulation, CD4+ and CD8+ T cells secreted IFN-γ [60]. Expression of IFN-γ by T cells suggests that DCs modified to express TAA may generate a potent helper T cell-1 (Th1)-type immune response. DCs modified with liposome complexes carrying another TAA gene, gp100, induced a strong and effective immune response against autologous tumours expressing gp100 and this response also depended on both CD4+ and CD8+ T cells. Moreover, this strategy was far more effective than immunisation of mice with a naked plasmid encoding the gp100 protein [23]. Protection of animals after administration of DCs expressing TAA requires the presence of CD4+ and CD8+ T cells during both the priming and challenge phases. In the study of Metharom and colleagues, DCs modified to express the mTRP-2 gene, a tumour rejection antigen for B16 melanoma, cured four of seven mice with pre-established tumours [61]. In another study, DCs transduced with an adenoviral vector encoding a model antigen, β-galactosidase (β-gal), have been shown to protect mice from a lethal tumour challenge. Mice that have been given an intravenous injection of murine colon carcinoma cells expressing β-gal developed pulmonary metastases within several days. However, mice preimmunised with DCs expressing the β-gal antigen were fully protected. In the same study, mice with pre-established lung metastases after administration of β-gal-modified DCs survived significantly longer than untreated control animals [62]. The antitumour immunity of animals vaccinated with DCs modified with the β-gal gene was long-lasting and was shown to be fully effective even at 300 – 400 days postimmunisation [63]. In order to improve the efficacy of immunisation with DCs modified to express TAAs, Kaplan and colleagues have tested a combination of two DC populations expressing gp100 and TRP genes in mice with pre-established subcutaneous melanomas. Such a combinatory therapy resulted in an enhanced inhibition of tumour growth as compared with administration of either DC population alone [38].

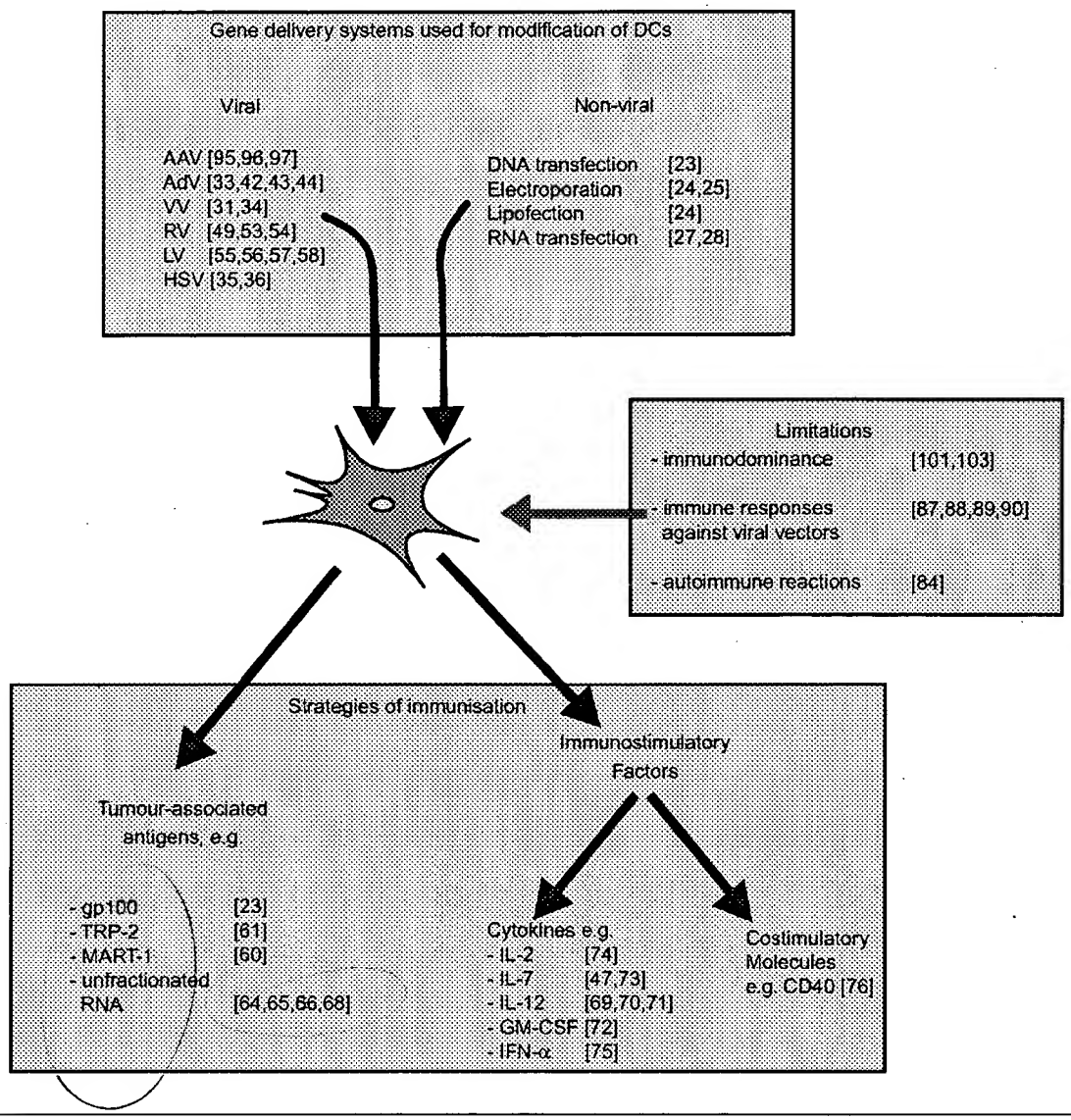

Figure 1. Cancer treatment strategies based on gene modified DCs.

AAV: Adeno-associated virus-based vectors; AdV: Adenoviral vectors; GM-CSF: Granulocyte macrophage-colony stimulating factor; HSV: Herpes simplex virus-based vectors; LV: Lentiviral vectors; MART: Melanoma antigen recognised by T cells; RV: Retroviral vectors; TRP: Tyrosinase related protein; VV: Vaccinia virus-based vectors.

Not all cancer cells express the well known, tumour-specific TAAs or may even express mutated versions of these. Thus, tumours may evade immune response induced by DCs modified to express the defined TAAs. However, modification of DCs with the entire cancer cell-derived genetic material may enable them to present different, even previously uncharacterised TAAs to immune cells and to stimulate polyclonal, cancer-specific CTLs [64-66]. DCs pulsed with unfractionated RNA derived from tumour cells expressing the model antigen OVA (chicken ovalbumin) have been shown to stimulate potent, primary CTL responses *in vivo* and to induce protection of vaccinated animals from a subsequent challenge with OVA-expressing cancer cells [28]. Immunisation of mice with DCs modified with RNA derived from poorly immunogenic, highly metastatic B16/F10 melanoma cells induced protection against CNS tumour challenge [67]. The efficacy of such a strategy of vaccination was at least equal as compared to immunisation with B16 tumour cells secreting GM-CSF [67]. Zhang *et al.* vaccinated mice with as few as 4 x 10^4 DCs loaded with tumour RNA and demonstrated:

- tumour-specific CTL activity
- protection of immunised animals against tumour rechallenge
- reduction in pulmonary metastases
- prolonged survival in the 3LL and B16 tumour models [27]

Heiser and colleagues have recently published results from a Phase I clinical trial of metastatic prostate cancer patients vaccinated with PBMC-derived DCs modified with mRNA

encoding prostate-specific antigen [68]. Repeated vaccinations with escalating doses of RNA-modified DCs (up to 5×10^7) decreased serum prostate-specific antigen velocities in six of seven patients and were associated with temporary complete clearance of blood-born circulating cancer cells in three of seven patients.

5.2 Dendritic cells modified with genes encoding immunostimulatory factors

Induction of antigen-specific T cell-mediated immune responses by DCs depends not only on presentation of antigens in the context of MHC complexes, but also on two further signals: costimulatory molecules (CD40, CD80, CD86) and cytokines (IL-12, IL-2, IL-10, TNF-α, IL-1β and IL-6). Multiple studies have been carried out in order to evaluate the effectivity of DCs modified with genes encoding immunostimulatory proteins in the induction of tumour-specific immune responses.

DCs modified to express IL-12 have been shown to induce a potent antitumour immune response [69-71]. In a murine model of colon cancer, intratumoural administration of DCs adenovirally transduced with the IL-12 gene inhibited growth of subcutaneous tumours. This effect was associated with an increased infiltration of tumours with CD4+ and CD8+ T cells and with polarisation of an immune response toward Th1/Tc1 cells [69]. In another study in three weakly immunogenic tumour models, intratumoural administration of bone marrow-derived IL-12-expressing DCs resulted in a complete regression of established tumours. The induced immune response was associated with the production of IFN-γ by Th cells [70]. In addition, in a murine model of neuroblastoma, the intratumoural injection of DCs modified to express IL-12 induced a complete regression of tumours within 3 weeks. This effect was also associated with decreased apoptosis of tumour infiltrating cells [71].

Transfection of immature DCs with a GM-CSF gene did not increase the immunostimulatory capacity of DCs, as demonstrated by analysis of MHC and costimulatory molecule expression as well as by antigen presentation to T cells *in vitro*. However, when administered *in vivo*, GM-CSF-expressing DCs demonstrated an enhanced migration into lymph nodes and induced a more potent immune response against different antigens tested as compared to unmodified DCs [72].

DCs transduced with a retroviral vector carrying an IL-7 gene increased in an autologous mixed lymphocyte reaction (MLR) T cell proliferation by a factor of 2 and in allogenic MLR by a factor of 2.7, as compared with unmodified DCs [47]. Miller and colleagues analysed the effect of intratumoural administration of DCs modified to express IL-7 [73]. In two murine lung cancer models, DCs transduced with an adenovirus carrying IL-7 gene induced an extremely potent and long-lasting antitumoural immune response comparable to intratumoural administration of an adenovirus encoding IL-7. However, mice that rejected tumours after administration of the genetically modified DCs were totally protected from a subsequent tumour rechallenge, whereas, only 20 – 25% of animals treated with adenovirus encoding IL-7 survived the rechallenge.

In a few studies, DCs modified simultaneously with genes encoding TAA and cytokines have been shown to induce potent antigen-specific antitumour immune response. In a study by Nakamura and colleagues, DCs transduced with an adenoviral vector carrying GM-CSF and gp70 (murine colon carcinoma antigen) genes have been shown to be superior in the induction of antitumour immune responses to DCs expressing exclusively the gp70 gene. Secretion of GM-CSF by transduced DCs enhanced the expression of CCR7 on DCs, which is crucial for an effective trafficking of DCs towards secondary lymphoid organs. Human DCs simultaneously expressing IL-2 and a MUC-1 (mucin-1) antigen have also been shown to effectively stimulate proliferation of autologous lymphocytes in MLRs [74]. Similarly, Tuting *et al.* have modified human monocyte-derived DCs with genes encoding malignant melanoma antigens (Mage-1, Mage-3, MART-1/Melan A, pMel-17/gp100 or tyrosinase). Cotransduction of those DCs expressing TAA with genes encoding IL-12 or IFN-α resulted in a strong activation of antigen-specific CTLs and polarisation of the immune response toward Th1 type cells [75].

Another strategy of modifying DCs with genes encoding immunostimulatory molecules was tested by Kikuchi and colleagues [76]. Murine bone marrow-derived DCs have been transduced with an adenoviral vector encoding CD40L protein. CD40L, usually expressed by CD4+ T cells, interacts with a CD40 located on the surface of DCs [76]. Several recent reports suggest that DCs cannot stimulate CTLs directly unless they are first stimulated via CD40 [77-79]. CD40 triggering by CD4+ T cells results in an upregulation of adhesion and costimulatory molecules and production of several inflammatory cytokines and chemokines such as IL-12 and MIP-1α [80-82].

Intratumoural injection of CD40L-expressing DC into B16 and CT26 tumours induced a strong inhibition of tumour growth and significantly extended survival of treated animals, as compared to unmodified DCs. Moreover, splenocytes obtained from mice treated with CD40L-expressing DCs were able to transfer tumour-specific immunity to naive recipients [76].

6. Expert opinion

Genetically modified DCs have been shown to be effective in the induction of antigen-specific immune responses both *in vitro* and *in vivo*. The relatively uncomplicated techniques of DC generation and continuous development of gene transfer technologies have made possible the genetic modification of both bone marrow-derived and PBMC-derived DCs. Although several cancer patients have already been vaccinated with genetically modified DCs, there are still some concerns associated with this strategy. Administration of DCs modified with the whole tumour-derived RNA, or

expressing TAAs that are also shared by normal cells, may lead to autoimmune reactions. Ludewig and colleagues demonstrated that DC-based vaccination of transgenic mice against tumour cells expressing an antigen also shared by cells in particular organs (cardiomyocytes or pancreatic β-islet cells) induced eradication of implanted tumours. However, it was also associated with fatal, destructive autoimmune reactions resulting in myocarditis, arteritis, dilated cardiomyopathy or diabetes [84].

Additional concerns are also associated with the use of viral vectors. The lentiviral vectors seem to be one of the most potent gene delivery vehicles for purposes of genetic modification of DC precursors. However, since they are based on a deadly pathogen, every single concern must be cleared before they are moved from laboratory benches to clinics. Integration of a retroviral vector with host genome may also induce expression of silenced oncogenes by promoters located within long-terminal repeat sequences.

In conclusion, genetically modified DCs are a very promising strategy of cancer therapy. However, there is still a huge need for identification of additional, unique TAAs and for further exploration of the cytokine networks. These elements seem to be crucial for construction of the next generations of safe, effective and feasible DC-based cancer vaccines.

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Affiliation

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DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 109

Genetically modified dendritic cells - A new, promising *cancer* treatment strategy?

...Due to the priming of antigen-specific immune responses mediated by CD4+ and CD8+ lymphocytes, DCs are crucial for the induction of adaptive immunity against *cancer*. Therefore, vaccination of *cancer* patients with DCs presenting tumour-associated antigens (TAAs) have been believed to be a promising anticancer strategy. Multiple clinical trials have been carried out in order to evaluate the safety and efficacy of *cancer* vaccines based on antigen-pulsed DCs. However, pulsing of DCs with particular peptides has several disadvantages: i) short-time duration of antigen-major histocompatability complex...

...proteins have been shown to be effective in the induction of antitumour immune responses. Based on these encouraging results, a first clinical trial of prostate *cancer* patients vaccinated with gene modified DCs has recently been initiated. In this article, methods used for genetic modification of DCs and anticancer vaccination strategies based on *genetically* *modified* DCs are reviewed.
DRUG DESCRIPTORS:
tumor antigen; *cancer* vaccine; peptide; major histocompatibility antigen; epitope
MEDICAL DESCRIPTORS:
**cancer* therapy; *DNA modification; *dendritic cell vaccination; *cancer* patient; safety; gene transfer; *cancer* immunization ; antineoplastic activity; prostate *cancer*--therapy--th; cell line; Vaccinia virus; virus vector; adenovirus vector; Herpes simplex virus; retrovirus vector; lentivirus vector; Adeno associated virus; immunostimulation; *cancer* genetics; human; clinical trial; *review*
SECTION HEADINGS:
016 *Cancer*
022 Human Genetics
026 Immunology, Serology and Transplantation

6/3,K/4 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

07633907 EMBASE No: 1999118833
Immunization strategies in advanced melanoma
Schadendorf D.
D. Schadendorf, Department of Dermatology, Clinics Mannheim, University of Heidelberg, Theodor Kutzer Ufer 1, 68135 Manheim Germany
Biogenic Amines (BIOG. AMINES) (Netherlands) 1999, 15/1 (53-72)
CODEN: BIAME ISSN: 0168-8561
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 52

Gene transfer of cytokine genes such as IL-2, IL-4, IL-7, IL-12, IFN-gamma, TNF-alpha and GM-CSF into *tumor* cells were shown to be able to induce a systemic anti-*tumor* immune response. Based on the animal *tumor* models, a number of clinical protocols have been developed to treat *cancer* patients with cytokine gene-modified *tumor* cells. Clinical phase I studies were initiated with autologous melanoma cells being *transfected* either with both chains of the IL-12 gene or with the IL-7 gene. Evaluation of the first 10 patients immunized with autologous, IL...

...the feasibility of such an approach, however, no major clinical response was achieved. Furthermore, peripheral blood lymphocytes were found to contain an increased number of *tumor*-reactive T-cells after immunization. The magnitude of the T-cell reactivity was found to be highly associated

with the patients' Karnof[redacted]y-index and recall...

...were immunized with autologous IL-12 gene modified melanoma cells. Clinically, there was no major toxicity except for mild fever. Post-vaccination, the number of *tumor*-reactive proliferative as well as cytotoxic T-cells was significantly increased in 2 patients (up to 15-fold). Two patients developed delayed type hypersensitivity (DTH...

...gene-modified melanoma cells induced, even in far advanced/terminally ill patients, immunological changes which can be interpreted as an increased antitumor immune response. Since *dendritic* *cells* (DC) can now easily be generated from different sources including peripheral blood, these cells can either be used after pulsing with peptides or after transfection with a *tumor* antigen for vaccination of *cancer* patients. The implication for vaccine design is that DC would be more potent than *tumor* cells as immunogens. In a clinical pilot study, DC were generated in the presence of GM-CSF and IL-4 and were pulsed with *tumor* lysate or a cocktail of peptides known to be recognized by CTLs depending on the patient's HLA haplotype. Sixteen patients with advanced melanoma were immunized on an outpatient basis. Vaccination was well tolerated. *Multiple* peptides were used to diminish the chances of immune escape in a given patient. In 4 patients, *tumor* lysate was used instead of peptide as source of *tumor* antigen. Significant DTH-reactivity against DC pulsed with melanoma-associated peptides was observed in 5 patients with 4 of these patients demonstrating a major clinical...
DRUG DESCRIPTORS:
cytokine; interleukin 2; interleukin 4; interleukin 7; interleukin 12; gamma interferon; *tumor* necrosis factor alpha; granulocyte macrophage colony stimulating factor; *tumor* antigen; peptide
MEDICAL DESCRIPTORS:
*immunization; *melanoma--etiology--et; *melanoma--therapy--th; *advanced *cancer*--etiology--et; *advanced *cancer*--therapy--th
gene transfer; immune response; *tumor* cell; genetic transfection; clinical protocol; *cancer* patient; safety; treatment outcome; peripheral lymphocyte; t lymphocyte; delayed hypersensitivity; fever--complication--co ; dendritic cell; cell lysate; HLA typing; skin metastasis--complication --co; remission; soft tissue; lung metastasis--complication--co; pancreas; human; clinical article; *review*
SECTION HEADINGS:
  013 Dermatology and Venereology
  016 *Cancer*
  022 Human Genetics
  026 Immunology, Serology and Transplantation
?ds

Set	Items	Description
S1	2837	((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (W) CELL-?)) (S) (ARRAY OR MULTIPLE OR POLYEPITOPE?)
S2	158	S1 (S) (TRANSFECTED OR TRANSFORMED OR (GENETICALLY (W) MODIFIED))
S3	0	S2 AND (DIFFERENTIAL (W) (DISPLAY OR SCREENING OR EXPRESSION))
S4	78	S2 AND (TUMOR OR CANCER)
S5	36	RD (unique items)
S6	4	S5 AND REVIEW

?s s5 not py>1998
36 S5
6831362 PY>1998
S7 11 S5 NOT PY>1998
?t s7/3,k/all

7/3,K/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11350173 98230454 PMID: 9570527
Autologous human monocyte-derived dendritic cells genetically modified to

express melanoma antig[redacted] elicit primary cytotoxic T[redacted]1 responses in vitro: enhancement by cotransfection of genes encoding the Th1-biasing cytokines IL-12 and IFN-alpha.

Tuting T; Wilson C C; Martin D M; Kasamon Y L; Rowles J; Ma D I; Slingluff C L; Wagner S N; van der Bruggen P; Baar J; Lotze M T; Storkus W J

Department of Surgery, University of Pittsburgh School of Medicine, University of Pittsburgh Cancer Institute, PA 15261, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Feb 1 1998, 160 (3) p1139-47, ISSN 0022-1767 Journal Code: 2985117R

Contract/Grant No.: CA57653; CA; NCI; CA57840; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

... study we have evaluated the feasibility of DNA vaccination for the induction of CTL reactivity to five different melanoma Ags in vitro. Cultured, monocyte-derived *dendritic* *cells* (DC) were transiently *transfected* with plasmid DNA encoding human MART-1/Melan-A, pMel-17/gp100, tyrosinase, MAGE-1, or MAGE-3 by particle bombardment and used to stimulate autologous PBMC responder T cells. CTL reactivity to these previously identified melanoma Ags was reproducibly generated after two or three stimulations with *genetically* *modified* DC. Co-ordinate transfection of two melanoma Ag cDNAs into DC promoted CTL responders capable of recognizing epitopes from both gene products. Coinsertion of genes encoding the Th1-biasing cytokines IL-12 or IFN-alpha consistently enhanced the magnitude of the resulting Ag-specific CTL reactivity. Importantly, DC *transfected* with a single melanoma Ag cDNA were capable of stimulating Ag-specific CTL reactivity restricted by *multiple* host MHC alleles, some of which had not been previously identified. These results support the inherent strengths of gene-based vaccine approaches that do not require prior knowledge of responder MHC haplotypes or of relevant MHC-restricted peptide epitopes. Given previous observations of in situ *tumor* HLA allele-loss variants, DC gene vaccine strategies may elicit a greater diversity of host therapeutic immunity, thereby enhancing the clinical utility and success of...

...; Glycoproteins--genetics--GE; Membrane Glycoproteins--immunology--IM ; Monocytes--immunology--IM; Mutagenesis, Insertional--immunology--IM; Neoplasm Proteins--genetics--GE; Neoplasm Proteins--immunology--IM; Peptides--immunology--IM; *Tumor* Cells, Cultured; Vaccines, DNA --immunology--IM

7/3,K/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11255558 98133163 PMID: 9472560

Multicomponent gene therapy vaccines for lung *cancer* : effective eradication of established murine tumors in vivo with interleukin-7/herpes simplex thymidine kinase-transduced autologous *tumor* and ex vivo activated dendritic cells.

Sharma S; Miller P W; Stolina M; Zhu L; Huang M; Paul R W; Dubinett S M

Department of Medicine, UCLA School of Medicine, USA.

Gene therapy (ENGLAND) Dec 1997, 4 (12) p1361-70, ISSN 0969-7128 Journal Code: 9421525

Contract/Grant No.: CA09120; CA; NCI; CA71818; CA; NCI; HL07014; HL; NHLBI; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Multicomponent gene therapy vaccines for lung *cancer* : effective eradication of established murine tumors in vivo with interleukin-7/herpes simplex thymidine kinase-transduced autologous *tumor* and ex vivo

activated dendritic cells

Multiple antitumor modalities may be necessary to overcome lung *tumor* -mediated immunosuppression and effectively treat non-small cell lung *cancer* (NSCLC). To evaluate a multimodality gene therapy approach for control of local *tumor* growth, a weakly immunogenic murine alveolar cell carcinoma, L1C2, was transduced with either the interleukin-7/hygromycin-herpes simplex thymidine kinase (IL-7/HyHSVtk) internal...

...gene. Of the many cytokines available for gene transfer, IL-7 was chosen for these studies because it both stimulates CTL responses and down-regulates *tumor* production of the immunosuppressive peptide TGF-beta. Following selection in hygromycin, IL-7 transduction was confirmed by ELISA. Clones produced 1.25 to 10 ng of IL-7/ml/10(6) cells per 24 h. In vitro, *genetically* *modified* *tumor* cells were significantly more sensitive to ganciclovir (GCV) than unmodified parental *tumor* cells. The in vivo growth of ex vivo modified L1C2 cells was evaluated. There was a dose-response relationship between the amount of IL-7 secreted in vitro and the growth of *genetically* *modified* murine *tumor* in vivo. Transduced *tumor* cells regressed in mice following GCV therapy. Although ex vivo gene modification of *tumor* cells led to complete resolution of the *tumor* following implantation in vivo, IL-7 and HSVtk gene modified *tumor* cells were not effective in treating established parental tumors. However when 5 x 10(5) bone marrow-derived, in vitro activated *dendritic* *cells* (DC) were administered in combination with transduced *tumor* and GCV, 5 day old established tumors were eradicated in 80% of mice. These studies suggest that multicomponent vaccines may facilitate improved host responses by...

...; IM; Dendritic Cells--immunology--IM; Ganciclovir--therapeutic use --TU; Lung Neoplasms--immunology--IM; Mice; Mice, Inbred BALB C; Simplexvirus--enzymology--EN; Thymidine Kinase--genetics--GE; *Tumor* Cells, Cultured

7/3,K/3 (Item 3 from file: 155)

10260412 96062042 PMID: 7589089

Major histocompatibility complex class II-associated peptides determine the binding of the superantigen toxic shock syndrome toxin-1.

von Bonin A; Ehrlich S; Malcherek G; Fleischer B
Bernhard-Nocht-Institut for Tropical Medicine, Hamburg, Germany.

European journal of immunology (GERMANY) Oct 1995, 25 (10) p2894-8, ISSN 0014-2980 Journal Code: 1273201
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

... and interact with variable parts of the T cell antigen receptor (TCR) beta-chain. Cross-linking the TCR with MHC class II molecules on the *antigen*-*presenting* *cell* by the superantigen leads to T cell activation that plays an essential role in pathogenesis. Recent crystallographic data have resolved the structure of the complexes...

... peptides by employing variants of T2 mutant cells deficient in loading of peptides to MHC class II molecules as superantigen-presenting cells. On HLA-DR3-*transfected* T2 cells, presentation of TSST-1, but not SEB, was dependent on HLA-DR3-associated peptides. Thus, although these superantigens can be recognized in the context of *multiple* MHC class II alleles and isotypes, they clearly bind to specific subsets of MHC molecules displaying appropriate peptides.

...; IM; Lymphocyte Activation; Macromolecular Systems; Molecular Sequence Data; Peptide Fragments--immunology--IM; Peptide Fragments --metabolism--ME; Protein Binding; Superantigens--immunology--IM; T-Lymphocytes--metabolism--ME; *Tumor* Cells, Cultured

7/3,K/4 (Item 1 from file: 159)
DIALOG(R)File 159:Cancerlit

02231764 PMID: 96606684
***Tumor* cells transfected with B7-1 + IL-12 CDNA induce lasting protective immunity (Meeting abstract).**
Ksander; Podack; Chen
Dept. of Ophthalmology, Harvard Medical School, Boston, MA 02114
FASEB J 1995, 9 (3), ISSN 0892-6638
Document Type: MEETING ABSTRACTS
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

***Tumor* cells transfected with B7-1 + IL-12 CDNA induce lasting protective immunity (Meeting abstract).**
Successful activation of specific T cells requires *multiple* costimulatory signals provided by *antigen* *presenting* *cells*; B7-1 which stimulates differentiation of precursor cytotoxic T cells, and IL-12 which stimulates differentiation of precursor T helper cells to become Th1 type cells. In an effort to increase the immunogenicity of *tumor* cells and activate *tumor*-specific T cells, we *transfected* P815 cells with CDNA for either B7-1 and/or IL-12 p35 and p40 chains. Untransfected P815 cells grow progressively and fail to induce *tumor*-specific T cells when injected into the flank of syngeneic mice. Expression of B7-1 alone results in the elimination of the primary *tumor* and induction of *tumor*-specific cytotoxic T cells. However, immunity is transient and only slows the growth of a secondary *tumor* challenge (100% *tumor* incidence). Similarly, mice immunized with transfectants that secrete IL-12 alone also failed to induce protective immunity to a second *tumor* challenge (100% incidence). By contrast, mice immunized with transfectants expressing B7-1 and secreting IL-12 were protected from a second *tumor* challenge (20% *tumor* incidence). We conclude that expression of B7-1 and secretion of IL-12 by P815 *tumor* cells induces a long-lasting and protective memory T cell response, and we suspect that this results from the induction of both cytotoxic T cells...

7/3,K/5 (Item 2 from file: 159)
DIALOG(R)File 159:Cancerlit

02230296 PMID: 96604719
Immunity to epithelial tumors and mucin-based vaccine design (Meeting abstract).
Finn
Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh Sch. of Medicine, Pittsburgh, PA 15261
Proc Annu Meet Am Assoc Cancer Res 1995, 36, ISSN 0197-016X
Document Type: MEETING ABSTRACTS
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

Mucin, encoded by the MUC-1 gene, is a transmembrane molecule which consists of *multiple* tandem repeats of a 20 amino acid sequence highly glycosylated by O-linked sugars. Both normal and malignant epithelial cells produce mucin, however, the *tumor* specificity of anti-mucin antibodies has been attributed to incomplete glycosylation of mucin by malignant cells. The importance of mucins as *tumor*-associated antigens has recently been increased by our finding that cytotoxic T lymphocytes (CTL) can recognize mucin produced by malignant and not by normal cells. We constructed several plasmid vectors which allow mucin production in different cell types, including autologous *antigen* *presenting* *cells*

(APC), such as B cells and *dendritic* *cells*. EBV immortalized B cells from *cancer* patients, *transfected* with mucin cDNA, provide an unlimited supply of autologous, mucin-bearing cells to use both as stimulators and as targets to detect anti-mucin immunity. We have shown in vitro that transfection of autologous B cells with mucin confers upon them *tumor* antigen presenting ability. In chimpanzees we have successfully elicited mucin specific T cell responses by immunizing with autologous B cells *transfected* with MUC1. It is attractive to postulate that these transfectants may be a vaccine of choice, capable of stimulating effective helper as well as cytotoxic T cell responses. We have established a reproducible system for growing human as well as chimpanzee *dendritic* *cells* and for their transduction with a retroviral vector carrying MUC1 cDNA. The mucin polypeptide core consists of many perfectly conserved, tandemly repeated 20 amino acids...

... vitro, but that the synthetic peptide corresponding to five tandem repeats has that capacity. The long peptide has allowed us to determine that 10% of *cancer* patients make antibodies directed to the mucin tandem repeat core, and to the same epitope detected by mouse antibodies and human T cells. This long peptide is the first formulation of a mucin-based vaccine that we are currently testing in breast, pancreatic and colon *cancer* patients. The Phase I vaccine trial was initiated in December 1993 and closed in June 1994 when 60 patients had received the vaccine. All patients...

...response confirmed our previous in vitro observations and suggested that our vaccine peptide 105mer expresses the epitopes previously seen by the immune system of the *cancer* patients. In addition to the immunodominant epitope which is recognized directly and does not bind MHC, we have identified a 9aa long peptide from the...

7/3,K/6 (Item 3 from file: 159)
DIALOG(R)File 159:Cancerlit

02225639 PMID: 95609251
***Tumor* cells transfected with B7-1 + IL-12 cDNA induce lasting protective immunity (Meeting abstract).**
Chen; Podack; Ksander
Dept. of Ophthalmology, Harvard Medical School, Boston, MA 02114
Proc Annu Meet Am Assoc Cancer Res 1995, 36, ISSN 0197-016X
Document Type: MEETING ABSTRACTS
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

***Tumor* cells transfected with B7-1 + IL-12 cDNA induce lasting protective immunity (Meeting abstract).**
Successful activation of specific T cells requires *multiple* costimulatory signals provided by *antigen* *presenting* *cells*; B7-1 which stimulates differentiation of precursor cytotoxic T cells, and IL-12 which stimulates differentiation of precursor T helper cells to become Th1 type cells. To increase the immunogenicity of *tumor* cells, we *transfected* P815 cells with cDNA for either B7-1 and/or IL-12 p35 and p40 chains. Untransfected P815 cells grow progressively and fail to induce *tumor* -specific T cells when injected into the flank of syngeneic mice. Expression of B7-1 alone results in the elimination of the primary *tumor* and induction of *tumor*-specific cytotoxic T cells. However, immunity is transient and only slows the growth of a secondary *tumor* challenge (100% *tumor* incidence). Similarly, mice immunized with transfectants that secrete IL-12 alone also failed to induce protective immunity to a second *tumor* challenge (100% incidence). In contrast, mice immunized with transfectants expressing B7-1 and secreting IL-12 were protected from a second *tumor* challenge (20% *tumor* incidence). We conclude that expression of B7-1 and secretion of IL-12 by P815 *tumor* cells induces a long-lasting and protective memory T cell response, and we suspect that

this results from the in[redacted]tion of both cytotoxic T cells

7/3,K/7 (Item 4 from file: 159)
DIALOG(R)File 159:Cancerlit

02062429 PMID: 94699557
Mucin-based immunotherapy of breast *cancer* (Meeting abstract).
Finn
Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh Sch. of Medicine, Pittsburgh, PA 15261
Non-serial 1993, SBT93: Society for Biological Therapy, 8th Annual Scientific Meeting: Biological Therapy of Cancer--VIII. November 10-14, 1993, Nashville, TN, p. 34, 1993.,
Document Type: JOURNAL ARTICLE
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

Mucin-based immunotherapy of breast *cancer* (Meeting abstract).
One of the primary challenges to *tumor* immunologists in recent years has been to define antigens on tumors cells capable of stimulating the immune system, primarily the effector T cells. One of these molecules, first identified by monoclonal *tumor* specific antibodies, is breast mucin. Mucin consists of *multiple* tandem repeats of a 20 amino acid sequence which is highly glycosylated by O-linked sugars. Since both normal and malignant epithelial cells produce mucin, the *tumor* specificity of anti-mucin antibodies has been postulated to result from incomplete glycosylation of mucin by malignant cells. The importance of mucins as *tumor*-associated antigens has recently been increased by our finding that cytotoxic T lymphocytes (CTL) can recognize mucin. The epitope recognized by the CTL is present...

... can be manipulated by various gene transfer and biochemical techniques. We constructed several expression vectors which allow mucin production in different cell types, including autologous *antigen* *presenting* *cells*. Using mucin *transfected* B cells as APC we generated specific T cell lines and clones from breast *cancer* patients. Both syngeneic and allogeneic APC could stimulate mucin specific CTL and serve as their targets. T cell clones could be divided into the major...

... activity when mucin is not fully glycosylated. Our data suggest that altered post-translational modification of normal gene products can result in the expression of *tumor* specific epitopes, which can be utilized to induce an effective anti-*tumor* response, when presented on an immunizing cell of choice. These observations are being utilized to construct a mucin-based vaccine.

7/3,K/8 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

06918160 EMBASE No: 1997202622
Dendritic cells and tumour rejection immune response
CELLULES DENDRITIQUES ET REPONSE IMMUNE DE REJET TUMORAL
Bernard J.; Kochman S.
Dr. J. Bernard, Unite de Therapie Cellulaire, Institut Jean-Godinot, 1, Avenue du General-Koenig, 51056 Reims Cedex France
Revue Francaise d'Allergologie et d'Immunologie Clinique (REV. FR. ALLERGOL. IMMUNOL. CLIN.) (France) 1997, 37/3 (271-277)
CODEN: RFAIB ISSN: 0335-7457
DOCUMENT TYPE: Journal; Conference Paper
LANGUAGE: FRENCH SUMMARY LANGUAGE: FRENCH; ENGLISH
NUMBER OF REFERENCES: 27

...destroys the tumour [redacted]thout affecting normal tissues. [redacted]his response is mediated by CD8+ cytotoxic T lymphocytes at the end of a complex process which involves *antigen*-*presenting* *cells* and CD4sup + helper T lymphocytes. The absence of effective response against *cancer* cells in the patient represents an obvious failure to the immune surveillance. Presentation of tumour antigens to T lymphocytes appears to be one of the deficient steps explaining this immunological escape of the tumour. The remarkable immunogenic properries of *dendritic* *cells* have led to their use as tumour *antigen* *presenting* *cells* in order to trigger immune rejection responses. All of the experimental data obtained to date constitute the basis for a concept of adjuvant immunotherapy. The strategy consists of producing *dendritic* *cells* from a patient, to sensitize them ex vivo with the targeted tumour antigen, then to reinject them in order to generate an effective immune response against the residual disease. Despite an encouraging preliminary clinical trial, the *multiple* parameters of anticancer immunization have yet to be evaluated before these experimental results can be *transformed* into clinical efficacy.
DRUG DESCRIPTORS:
*cd4 antigen--endogenous compound--ec; *cd8 antigen--endogenous compound --ec; **tumor* antigen--endogenous compound--ec
MEDICAL DESCRIPTORS:
**cancer* immunotherapy; *dendritic cell; *t lymphocyte; **tumor* rejection
SECTION HEADINGS:
005 General Pathology and Pathological Anatomy
016 *Cancer*
026 Immunology, Serology and Transplantation

7/3,K/9 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

03536798 EMBASE No: 1987053734
Presentation of soluble antigen to human T cells by products of multiple HLA-linked loci: Analysis of antigen presentation by a panel of cloned, autologous, HLA-mutant Epstein-Barr virus-transformed lymphoblastoid cell lines
Chen B.P.; DeMars R.; Sondel P.M.
Department of Human Oncology, University of Wisconsin, Madison, WI 53792 United States
Human Immunology (HUM. IMMUNOL.) (United States) 1987, 18/1 (75-91)
CODEN: HUIMD
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

Epstein-Barr virus-*transformed* human B lymphoblastoid cell lines (EBV-LCL) can present soluble antigens to antigen-primed T lymphocytes. In this study, we used HLA antigen-loss mutants...

...721) to demonstrate that the presentation of a soluble antigen from Candida albicans (CAN) by EBV-LCL to primed T cells can be restricted by *multiple* HLA determinants. Haplotype-deletion mutants that contained only the maternal or only the paternal HLA-haplotype were used to demonstrate the preferential role of autologous...

...Immunoselected mutants of LCL-721 showing a variety of distinct phenotypes that are deficient in HLA-DR, DQ, or DP antigen expression were tested as *antigen*-*presenting* *cells*. The antigen-presenting ability of these class II deficient EBV-LCL variants weakened with progressive loss of class II HLA determinants experssed on the cell surface. Our study, therefore, provides evidence for *multiple* HLA restriction determinants, including HLA-DR, DQ, and DP. Furthermore, LCL lacking all HLA-DR, DQ, and DP expression because of homozygous deletion of these...
SECTION HEADINGS:
026 Immunology, Serology and Transplantation
025 Hematology
022 Human Genetics

016 *Cancer*

7/3,K/10 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

03473743 EMBASE No: 1987226324
Tumour promotors but not initiators deplete Langerhans cells from murine epidermis
Halliday G.M.; Mac Carrick G.R.; Muller H.K.
Department of Pathology, University of Tasmania, Hobart 7001 Australia
British Journal of Cancer (BR. J. CANCER) (United Kingdom) 1987, 56/3 (328-330)
CODEN: BJCAA ISSN: 0007-0920
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

While *transformed* cells may be inhibited from growing into a tumour by an immune response mounted against tumour-associated-antigens presented via LC to T lymphocytes, depletion of the LC by promotors would abrogate this response, thus enabling the *transformed* cell to grow unhindered. However, this may not be the only effect of tumour promotors on anti-tumour immunity as TPA has also been shown...

...NK cell tumour cytotoxicity (Keller, 1979). In contrast to our findings with tumour promotors, tumour initiators had no effect on LC. Whether alteration of local *antigen*-*presenting* *cells* occurs in other models of chemical carcinogenesis which involve *multiple* steps, such as in the liver (Farber, 1984), is unknown, but such an investigation would determine if this is a requirement for tumour growth at...
DRUG DESCRIPTORS:
tumor promoter
SECTION HEADINGS:
013 Dermatology and Venereology
016 *Cancer*
052 Toxicology

7/3,K/11 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE

02835696 EMBASE No: 1985179655
Human T cell leukemia/lymphoma virus-infected antigen-specific T cell clones: Indiscriminant helper function and lymphokine production
Volkman D.J.; Popovic M.; Gallo R.C.; Fauci A.S.
Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205 United States
Journal of Immunology (J. IMMUNOL.) (United States) 1985, 134/6 (4237-4243)
CODEN: JOIMA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

...p24, and 4D12 not present in the uninfected clone. Prior to infection, the T cell clone responded to antigen stimulation in the presence of histocompatible *antigen*-*presenting* *cells* with proliferation and secretion of *multiple* lymphokines, including IL 2, B cell growth factor (BCGF), B cell differentiation factor (BCDF), and interferon-gamma (IFN-gamma). Following infection, the T cell clone...

...or antigen. Co-cultivation with any accessory cells regardless of histocompatibility resulted in increased proliferation and lymphokine production. IL 2 production by the HTLV-I-*transformed* cell, however, could not be detected. Similarly, the uninfected clone was able to provide

```
B cell help for Ig production only when stimulated with b    ...
SECTION HEADINGS:
  026  Immunology, Serology and Transplantation
  016  *Cancer*
  025  Hematology
  047  Virology
?ds

Set     Items   Description
S1       2837   ((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (W) CELL-
            ?)) (S) (ARRAY OR MULTIPLE OR POLYEPITOPE?)
S2        158   S1 (S) (TRANSFECTED OR TRANSFORMED OR (GENETICALLY (W) MOD-
              IFIED))
S3          0   S2 AND (DIFFERENTIAL (W) (DISPLAY OR SCREENING OR EXPRESSI-
              ON))
S4         78   S2 AND (TUMOR OR CANCER)
S5         36   RD (unique items)
S6          4   S5 AND REVIEW
S7         11   S5 NOT PY>1998
?s (array or multiple) (s) (antigen or antigens)
          77385  ARRAY
        1088772  MULTIPLE
        1098808  ANTIGEN
         714880  ANTIGENS
      S8   38901  (ARRAY OR MULTIPLE) (S) (ANTIGEN OR ANTIGENS)
?s s8 (s) ((antigen (w) presenting (w) cell?) or (dendritic (w) cell?))
Processing
Processing
Processing
          38901  S8
        1098808  ANTIGEN
         245395  PRESENTING
        8712137  CELL?
          39139  ANTIGEN(W)PRESENTING(W)CELL?
          89065  DENDRITIC
        8712137  CELL?
          45691  DENDRITIC(W)CELL?
      S9    1925  S8 (S) ((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC
                  (W) CELL?))
?s s9 (s) (transfected or transformed or infected or (genetically (w) modified))
           1925  S9
         142085  TRANSFECTED
         191036  TRANSFORMED
         521964  INFECTED
         140768  GENETICALLY
         468466  MODIFIED
          19839  GENETICALLY(W)MODIFIED
     S10   229  S9 (S) (TRANSFECTED OR TRANSFORMED OR INFECTED OR
                  (GENETICALLY (W) MODIFIED))
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           229  S10
        2133852  TUMOR
        2259658  CANCER
     S11    99  S10 AND (TUMOR OR CANCER)
?s s11 and (differential (w) (display or expression or screen or screening))
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         838831  DIFFERENTIAL
         157054  DISPLAY
        2040746  EXPRESSION
          86727  SCREEN
         536876  SCREENING
          38954  DIFFERENTIAL(W)(((DISPLAY OR EXPRESSION) OR SCREEN) OR
                  SCREENING)
     S12     0  S11 AND (DIFFERENTIAL (W) (DISPLAY OR EXPRESSION OR
                  SCREEN OR SCREENING))
?rd s11
...examined 50 records  (50)
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...completed examining records
S13 47 RD S11 (unique items)
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47 S13
6831362 PY>1998
S14 11 S13 NOT PY>1998 .
?s s14 not s7
11 S14
11 S7
S15 2 S14 NOT S7
?t s15/2,k/all

15/2,K/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11435290 98317971 PMID: 9655265

Induction of prostate *tumor*-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide.

Peshwa M V; Shi J D; Ruegg C; Laus R; van Schooten W C
Dendreon Corporation, Mountain View, California 94043, USA. mvpeshwa@dendreon.com

Prostate (UNITED STATES) Jul 1 1998, 36 (2) p129-38, ISSN 0270-4137 Journal Code: 8101368
Document type: Journal Article
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Subfile: INDEX MEDICUS; AIDS/HIV
Tags: Human; Male
Descriptors: *Acid Phosphatase--immunology--IM; *Antigen-Presenting Cells --immunology--IM; *CD8-Positive T-Lymphocytes--immunology--IM; *Peptide Fragments--immunology--IM; *Prostate--enzymology--EN; *Prostatic Neoplasms --immunology--IM; Acid Phosphatase--chemistry--CH; Cytotoxicity, Immunologic; Dendritic Cells--immunology--IM; HLA-A2 Antigen--metabolism --ME; Peptide Fragments--chemistry--CH
CAS Registry No.: 0 (HLA-A2 Antigen); 0 (Peptide Fragments)
Enzyme No.: EC 3.1.3.2 (Acid Phosphatase)
Record Date Created: 19980716
Record Date Completed: 19980716

Induction of prostate *tumor*-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide.

BACKGROUND: Most strategies in *cancer* immunotherapy are aimed at the induction of a strong cellular immune response against the *tumor*. Particularly, CD8+ T lymphocytes have been proven in multiple animal models to be critical for the eradication of solid tumors. METHODS: We used a population of peripheral blood-derived *antigen*-*presenting* *cells* (APC), containing *dendritic* *cells* (DC), to generate prostate *tumor* -specific CD8+ T cells. Selected peptides from prostatic acid phosphatase (PAP), a prostate tissue-specific *antigen*, were shown to bind HLA-A2. A high-affinity peptide was used to generate peptide-specific CD8+ cytolytic T lymphocytes (CTL) from the peripheral blood of healthy donors. RESULTS: The obtained PAP-peptide-specific CTL lysed peptide-coated target cells, vaccinia-*infected* target cells, and HLA-A2-positive prostate-*tumor* cells in vitro in an *antigen*-specific manner. CONCLUSIONS: Our results indicate that CTL precursors to the PAP gene product exist and could be potentially recruited to elicit an antitumor response. Thus, PAP is a suitable *antigen* for inclusion in prostate *cancer* vaccines.

15/2,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

variance/mean, or by comparing the average MEJC amplitude distribution skewness (Fig. 5a). This indicates that large spontaneous events are not due to the nonrandom coupling of multiple spontaneous release events by gated influx of calcium into the presynaptic terminal. However, we cannot discount the possibility that calcium fluxes from intracellular stores cause the nonrandom spontaneous release of multiple vesicles.

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Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs

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$CD8^+$ cytotoxic T lymphocytes (CTLs) mediate resistance to infectious agents and tumours. Classically, CTLs recognize antigens that are localized in the cytoplasm of target cells, processed and presented as peptide complexes with class I molecules of the major histocompatibility complex (MHC)[1]. However, there is evidence for an exogenous pathway whereby antigens that are not expected to gain access to the cytoplasm are presented on MHC class I molecules[2–6]. The most dramatic example is the *in vivo* phenomenon of cross-priming[7]: antigens from donor cells are acquired by bone-marrow-derived host antigen-presenting cells (APCs) and presented on MHC class I molecules. Two unanswered questions concern the identity of this bone-marrow-derived cell and how such antigens are acquired. Here we show that human dendritic cells, but not macrophages, efficiently present antigen derived from apoptotic cells, stimulating class I-restricted $CD8^+$ CTLs. Our findings suggest a mechanism by which potent APCs acquire antigens from tumours, transplants, infected cells, or even self-tissue, for stimulation or tolerization of CTLs.

Influenza A virus establishes a non-toxic infection in human dendritic cells (DCs)[8,9]. Once infected, these DCs are capable of eliciting virus-specific, recall CTL responses within 7 days. The CTLs generated are class I-restricted and kill virus-infected monocytes and peptide-pulsed target cells[8,10]. Compared with DCs, influenza-infected monocytes are poor stimulators of CTL responses[8] and undergo apoptosis[11,12]. We have exploited these observations to investigate the role of apoptosis in the generation of antigen that could be acquired by uninfected DCs.

DCs were prepared from peripheral blood precursors of HLA-$A2.1^+$ donors[13,14]. Uninfected DCs and syngeneic T cells were cocultured with influenza-infected syngeneic (Fig. 1a) or allogeneic (Fig. 1b) monocytes for 7 days. Influenza-specific CTLs were generated in these cocultures, suggesting that the DCs acquired antigen from the monocytes. The DCs and not the infected monocytes functioned as the antigen-presenting cell (APC), because the latter failed to stimulate CTLs in the absence of DCs, even at a stimulator:responder ratio of 1:2 (Fig. 1a, dashed lines). The CTL responses generated in these cocultures were as potent as those induced by influenza-infected DCs, and as few as 5×10^3 infected monocytes could charge uninfected DCs for the induction of robust CTLs (Fig. 1b). To confirm that antigen is transferred to and expressed directly by DC MHC class I products, we cocultured uninfected DCs and influenza-infected monocytes, and used the DCs as targets for influenza-specific CTLs (Fig. 1c). DCs cocultured with infected monocytes were recognized as efficiently as virus-infected DCs. Collectively, the results in Fig. 1 suggest that influenza antigen from infected monocytes gained access to MHC class I of the DC, that is, antigen from HLA-mismatched monocytes were cross-priming T cells through the DC.

It was important to exclude live virus as the agent responsible for the transfer of antigen to DCs. Influenza-infected monocytes produce only low levels of infectious virus before they undergo apoptosis[9,11]. Infection of 5×10^4 monocytes with a multiplicity of infection (MOI) of 2.0 would be expected to yield up to 1×10^5 infectious virions after a 24 h culture period. To prevent infection of DCs by monocyte-produced virus, experiments were done in human serum, which contains blocking anti-haemagglutinin antibodies. Indeed, the addition of influenza virus (10^4 infectious virions) directly into cultures containing uninfected DCs and T cells did not yield a CTL response (data not shown). Furthermore, <1 haemagglutination units (HAU) ml^{-1} was detected in the medium at 12 h, 24 h and 7 days of coculture.

The CTL responses were also not due to free peptide released by the dying monocytes, thereby charging class I molecules on DCs. Media from wells containing the infected monocytes were collected after an overnight culture and transferred to fresh wells containing T cells and uninfected DCs. Virus-specific CTLs were generated after a 7 day culture period (Fig. 2a, transfer). This CTL activity was abrogated if the medium was passed through a filter (0.45 μm pore size) before being added to T-cell–DC cultures (Fig. 2a, filter), suggesting that the antigenic material was neither live virus nor free peptide (as both would have passed through the filter). This was confirmed by sedimentation experiments (Fig. 2b). Media from wells containing infected monocytes were removed and spun at 250*g*. The antigenic material could be localized in the pellet, but not in the supernatant fraction. Notably, the pelleted material (which lacked detectable haemagglutinating activity) fully accounted for the CTL activity generated in direct transfer. These results are most consistent with the source of antigen being material derived from apoptotic cells.

The role of apoptosis in the transfer of antigen to the uninfected DC was therefore assessed. We first extended prior work[11], showing that influenza-infected monocytes undergo apoptosis as detected by annexin V binding, an early marker for programmed cell death (Fig. 3a, middle panel). In contrast, heat-inactivated (HI) influenza virus, which reliably reduces viral replication, failed to induce apoptosis in monocytes (Fig. 3a, bottom panel). However, DCs that are infected with HI influenza still stimulate potent influenza-specific CTL responses[10]. We employed this replication-deficient virus to probe the requirement for apoptosis in cross-priming. When media from wells containing the monocytes infected with HI influenza were collected after an overnight culture and transferred to fresh wells containing T cells and uninfected DCs, no influenza-specific CTLs were generated (Fig. 3b, 10 h transfer, HI Flu). In contrast, DCs exposed to media derived from live virus-infected cultures, which contained apoptotic cells, did elicit a CTL response (Fig. 3b, 10 h transfer, Live Flu). However, if the monocytes infected with HI influenza were allowed to undergo apoptosis spontaneously, as occurs during 7 days of culture[15], antigenic material was generated and virus-specific CTLs were induced (Fig. 3b, coculture, HI Flu). This data also argues against the possibility that live virus within the apoptotic cell is responsible for the transfer of antigenic material to the DCs.

To establish that apoptosis is the critical trigger for cross-priming, we used Z-VAD-CHO, an irreversible peptide inhibitor of caspase activity. Apoptosis of influenza-infected monocytes was blocked (determined by TUNEL), without affecting the expression of viral proteins (data not shown). Influenza-infected monocytes were cultured overnight in the presence of varying concentrations of Z-VAD. Media from these wells were then added to fresh wells containing T cells and uninfected DCs. At concentrations of Z-VAD which inhibited apoptosis of the infected monocytes, antigenic material was not transferred to the uninfected DCs, and virus-specific CTLs were not generated (Fig. 3c). We next compared apoptotic death to necrotic death for the generation of antigenic material. Influenza-infected 293 cells express influenza proteins after 10 h of infection but, unlike monocytes, do not undergo apoptosis. Apoptosis or necrosis was induced by ultraviolet B irradiation and hypotonic shock, respectively, and the cells were then cocultured with uninfected DCs and T cells for 7 days. CTL activity was measured using matrix peptide-pulsed T2 targets. Only the apoptotic cells and not the necrotic cells were capable of charging DCs with antigen (Fig. 3d). Apoptotic versus necrotic influenza-infected monocytes gave similar results (data not shown).

It was important to investigate the cellular requirements for generating CTLs through this pathway. When $CD4^+$ and $CD8^+$ subpopulations were purified at the end of the 7 day culture period, CTL activity was detected only in the $CD8^+$ fraction (data not shown). We next compared DCs to macrophages as potential mediators of this exogenous pathway. Uninfected HLA-A2.1$^+$

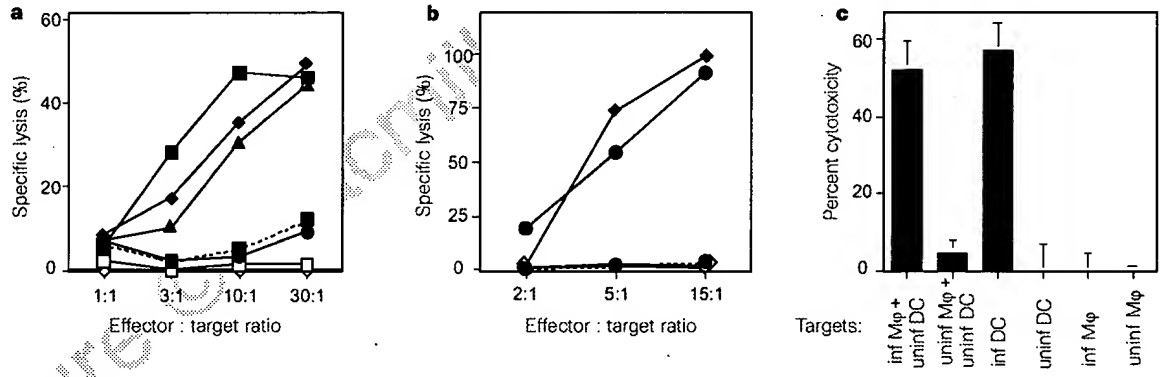


Figure 1 Dendritic cells acquire antigen from influenza-infected cells and induce class I-restricted CTLs. **a**, **b**, 8 to 10 h after infection with influenza virus, varying doses of syngeneic HLA-A2.1$^+$ monocytes (**a**) or allogeneic HLA-A2.1$^-$ monocytes (**b**) were added to cocultures of DCs and T cells. On day 7, cytolytic activity was tested using syngeneic influenza-infected macrophages (**a**) or T2 cells (a Tap$^{-/-}$, HLA-A2.1$^+$ cell line) pulsed with the immunodominant influenza matrix peptide[36] (**b**) as targets. Symbols: filled squares plus solid line 5×10^5 infected monocytes (inf Mϕ) + uninfected dendritic cells (uninf DCs); open squares 5×10^5 uninf Mϕ + uninf DCs; filled squares plus dotted line, 5×10^5 inf Mϕ, no DCs; filled triangles, 5×10^4 inf Mϕ + uninf DCs; filled circles plus solid line, 5×10^5 inf Mϕ + uninf DCs; open circles, 5×10^5 uninf Mϕ + uninf DCs; filled circles plus dotted line, 5×10^5 inf Mϕ, no DCs; filled diamonds, inf DCs; open diamonds, uninf DCs. **c**, Uninfected syngeneic DCs are cocultured with influenza-infected or uninfected allogeneic monocytes for 2 days before being used as targets for CTLs. Control targets included influenza-infected syngeneic DCs and influenza-infected allogeneic monocytes. Effector:target ratio = 45:1. Results are representative of 8 experiments and the values shown represent the mean from triplicate wells.

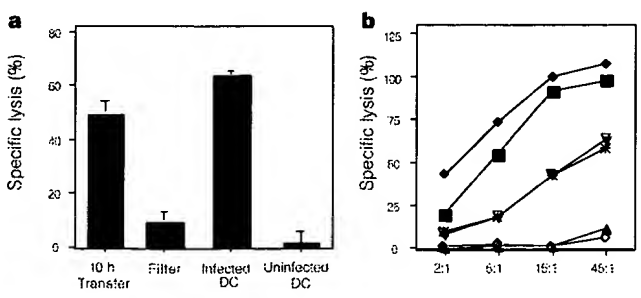


Figure 2 Antigen transfer is not due to live influenza virus or free peptide. **a**, 5×10^4 infected allogeneic HLA-A2.1$^-$ monocytes were cultured for 10 h, after which the media from these wells was collected. Media was either directly added to fresh wells containing HLA-A2.1$^+$ T-cells and DCs (10 h transfer) or first passed through a 0.45 μm (filter). Effector: target ratio = 30:1. **b**, 5×10^5 infected allogeneic monocytes were cultured for 10 h, after which T cells and DCs were added to the wells (squares). Alternatively, media from wells containing infected monocytes was removed and transferred to fresh wells containing T cells and DCs (asterisks). Other cultures were established in which the medium was first spun at 250*g* in a GH-3.8 Beckman rotor for 10 min. The resulting supernatant fraction (triangles) versus the pellet (inverted triangles) was then added to DC-T cell cocultures. Controls included T cells cultured with infected (filled diamonds) and uninfected (open diamonds) DCs. After 7 days, cytolytic activities were determined on T2 cells pulsed with matrix peptide. Results are representative of 3 experiments.

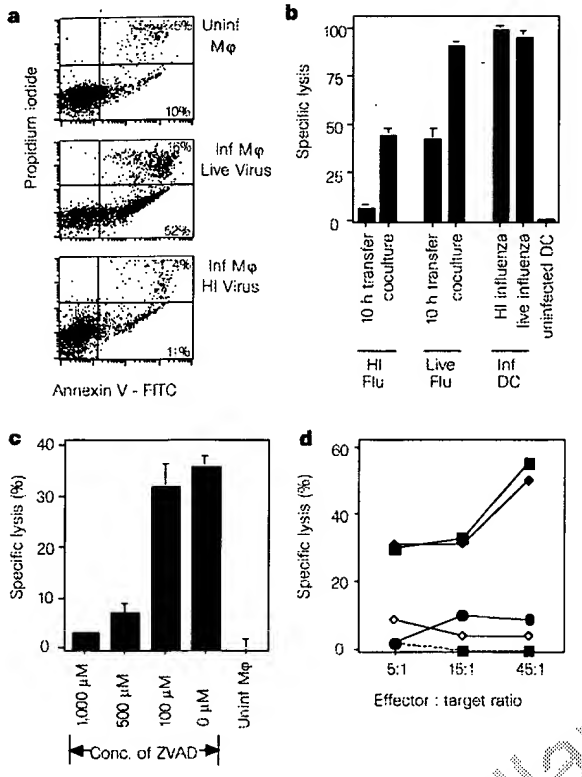


Figure 3 Apoptosis is required for delivery of antigen to DCs. **a**, Monocytes were infected with live or heat-inactivated influenza virus, cultured for 10 h and stained with annexin V – FITC (Kayima Biomedical Company, Seattle, WA) and propidium iodide. Early apoptotic cells are defined by the annexin V$^+$, propidium iodide$^-$ population. **b**, Allogeneic HLA-A2.1$^-$ monocytes were infected with live or heat-inactivated virus. After 10 h, media from wells containing infected monocytes was removed and added to fresh wells containing T cells and uninfected DCs (10 h transfer). Alternatively, the infected monocytes were directly cocultured with the T-cells and DCs (coculture). Heat-inactivated influenza and live influenza-infected DCs served as the positive controls for the experiment. After 7 days CTL activity was measured on matrix peptide-pulsed targets. **c**, Apoptosis of influenza-infected monocytes was inhibited using Z-VAD-CHO (Kayima Biomedical Company). 1×10^6 infected monocytes were exposed to varying doses of Z-VAD for 1 h, washed and cultured for 10 h. Media from these wells was then transferred to fresh wells containing 2×10^6 T cells and 6.67×10^5 DCs. Cytolytic activity was determined as in **b**. **d**, 293 cells, a human kidney epithelial cell line, were infected with influenza virus, and cultured for 10 h. Apoptosis was induced by UVB irradiation for 2 min[25]. Necrosis was achieved by incubating the 293 cells in a hypotonic solution for 30 min at 37 °C, after which all the cells incorporated trypan blue. Apoptotic or necrotic 293 cells were cocultured with T cells and uninfected DCs for 7 days. Cytolytic activity was determined as in **b**. Uninfected 293 cells failed to induce CTL activity (data not shown). Symbols: squares plus solid line, apoptotic inf 293 cells + uninf DCs; circles, necrotic inf 293 cells + uninf DCs; squares plus dotted line, apoptotic inf 293 cells, no DCs; filled diamonds, inf DCs; open diamonds, uninf DCs. The results are representative of 9 experiments.

macrophages or DCs and syngeneic T cells were cocultured with infected HLA-A2.1$^-$ monocytes for 7 days, after which cytolytic activity was measured. DCs but not macrophages were capable of stimulating influenza-specific CTLs (Fig. 4). Additionally, as increasing doses of syngeneic uninfected macrophages were introduced into cocultures containing uninfected DCs, T cells and infected allogeneic monocytes, the CTL activity was abrogated. Presumably, the macrophages act to sequester antigen from the DCs by efficiently engulfing the apoptotic cells and degrading the antigen.

To demonstrate that antigen was being processed intracellularly, DCs were pretreated with inhibitors of antigen presentation and

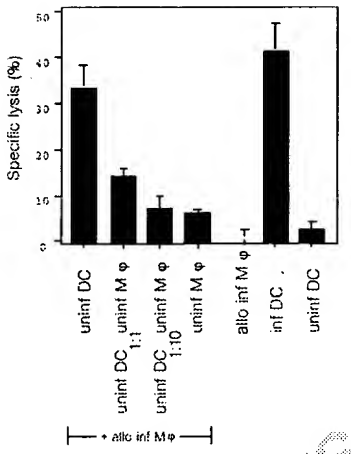


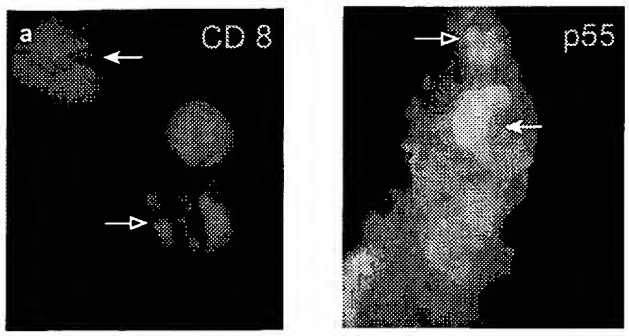
Figure 4 Dendritic cells and not macrophages are capable of cross-presenting apoptotic antigenic material. Uninfected HLA-A2.1$^+$ monocytes or DCs and mixtures of both were used as APCs, and cocultured with bulk syngeneic T cells and influenza-infected HLA-A2.1$^-$ monocytes. Infected HLA-2.1$^-$ DCs and infected HLA-2.1$^-$ monocytes served as positive and negative controls, respectively. Cytolytic responses were measured after 7 days. Effector:target ratio = 30:1.

used as targets for influenza-specific CTLs. Both ammonium chloride and Brefeldin A completely inhibited the DC's ability to present antigenic material derived from apoptotic monocytes (data not shown). Lactacystin, an irreversible inhibitor of the 26S proteosome[16], only partially blocked antigen presentation by the uninfected DCs (data not shown), suggesting that both classical and non-classical class I pathways are utilized for the presentation of exogenous antigen derived from apoptotic cells. We next documented the uptake of apoptotic cells by immunofluorescence and electron microscopy. Ten to twenty percent of the DCs contained fragmented or intact cell bodies in Fig. 5a. DCs were identified by the DC-restricted marker, p55, and apoptotic material was identified by the intense DAPI staining of pyknotic nuclei. This was confirmed by electron microscopy (Fig. 5b). About 20–25% of the DCs (identified by expression of CD83, data not shown) had vesicles containing apoptotic corpses with apparently intact plasma membranes. A recent report indicates that DCs associate with apoptotic cells through the vitronectin receptor $\alpha v\beta 3$, but fail to associate with opsonized particles and necrotic cells[17]. Further studies will be required to determine if DCs are actually phagocytosing and presenting apoptotic cells through this receptor.

Previous studies have shown that murine DCs have the capacity to present soluble antigens through an exogenous pathway, leading to the induction of MHC class I-restricted CTLs[5,18–20]. Following intravenous injection of allogeneic cells into rats, interdigitating DCs within lymph nodes were found to contain whole cells and cell fragments[21]. Here we present a physiologically relevant system which demonstrates that human DCs can acquire relevant antigens and stimulate MHC class I-restricted CTLs by phagocytosing apoptotic cells. Our studies provide the first evidence that apoptosis (but not necrosis) is required for the generation and packaging of immunogenic material for delivery to the DC. We believe this pathway accounts for the *in vivo* phenomenon of 'cross-priming', whereby antigens derived from tumour cells[22] or transplants[23] are presented by host APCs. Tolerance to tissue-restricted self antigens[24,25] may also depend upon apoptotic cell death (as occurs during development and normal cell turnover) followed by antigen presentation by DCs. Possibly, heat shock proteins within the apoptotic cells direct antigen into the class I MHC presentation pathway[4]. As a consequence of apoptosis, antigens within cells that lack costimulatory function for T cells can gain access to the potent DC system, thereby eliciting stimulatory or tolerogenic responses. This apoptosis-dependent pathway has the potential to be therapeutically manipulated to induce CTL responses *in vivo* to a variety of antigens including tumour and microbial antigens, and possibly to modulate autoimmune responses. □

Methods

Generation of mononuclear subsets. Peripheral blood mononuclear cells



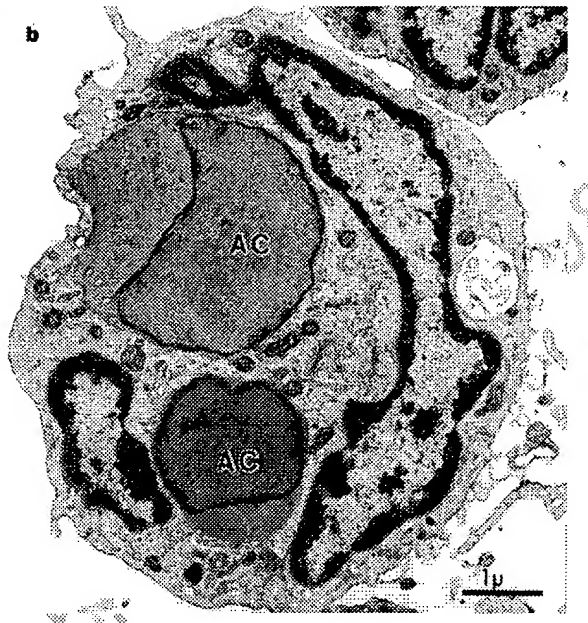


Figure 5 Dendritic cells engulf apoptotic monocytes. Influenza-infected monocytes and uninfected DCs were cocultured for up to 10 h. **a**, Cells were fixed with acetone and stained with anti-CD8 (isotype control) or anti-p55 followed by goat anti-mouse-FITC and incubation with DAPI (Sigma). The nuclei of the DCs (closed arrows) are lobulated and euchromatic as compared with the pyknotic, fragmented nucleus of the apoptotic cells (open arrows). $DAPI^+$ material from an apoptotic cell appears to be within the cytoplasm of a $p55^+$ DC. **b**, Electron microscopy revealed apoptotic material and apoptotic cells (AC) within the cytoplasm of glutaraldehyde-fixed DCs[25].

(PBMCs), T cells, and macrophages were prepared as previously described[4]. Dendritic cells were prepared from T-cell depleted PBMCs by culturing cells for 7 days in the presence of granulocyte and macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4), followed by 4 days in monocyte-conditioned medium[13,14].

Induction and detection of apoptosis. Monocytes were infected with influenza virus in serum-free RPMI. Cell death was assayed using the Early Apoptosis Detection kit (Kayima Biomedical). Briefly, cells are stained with Annexin V-FITC (Ann V) and propidium iodide (PI). Early apoptosis is defined by $Ann\ V^+/PI^-$ staining as determined by FACScan (Becton Dickinson). Cells from the 293 cell line were triggered to undergo apoptosis using a 60UVB lamp (Derma Control Inc.), calibrated to provide 2 mJ cm^{-2} s^{-1}.

Coculture of DCs with apoptotic cells. Monocytes from HLA-A2.1$^-$ donors were infected with live or heat-inactivated influenza virus. Live influenza virus (Spafas Inc.) was added at a final concentration of 250 HAU ml^{-1} (MOI of 0.5) for 1 h at 37 °C (ref. 8). Virus was heat-inactivated by treatment for 30 min at 56 °C before use[10]. After washing, cells were added to 24-well plates in varying doses. After 1 h contaminating non-adherent cells were removed and fresh media was added. Following a 10 h incubation at 37 °C, 3.3×10^3 uninfected DCs and 1×10^6 T cells were added to the wells.

Assay for virus-specific CTLs. After 7 days of culture, T cells were assayed for cytolytic activity using a conventional $Na^{51}CrO_4$ release assay[8]. The targets were either influenza-infected syngeneic monocytes or T2 cells (a $TAP^{-/-}$, HLA-A2.1$^+$, class II$^-$ cell line) pulsed for 1 h with 1 μM of the immunodominant influenza matrix peptide, GILGFVFTL[26,27]. Specific lysis was determined by subtracting the per cent killing of uninfected monocytes or unpulsed T2 cells. Influenza-infected DCs served as controls in all experiments in order to measure the donor's CTL responsiveness to influenza. Responses varied as a function of the individual's prior exposure to influenza. Background lysis ranged from 0–5% for the uninfected monocytes and 0–20% for the unpulsed T2 cells. Additionally, syngeneic DCs were used as targets where noted.

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11306170 98185398 PM[redacted] 9524710

Use of recombinant poxviruses to stimulate anti-melanoma T cell reactivity.

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CAS Registry No.: 0 (Antigens, Neoplasm); 0 (Genetic Vectors); 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II); 83869-56-1 (Granulocyte-Macrophage Colony-Stimulating Factor)

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BACKGROUND: *Dendritic* *cells* (DC) are potent professional *antigen*-*presenting* *cells* that can activate naive T lymphocytes and initiate cellular immune responses. As adjuvants, DC may be useful for enhancing immunogenicity and mediating *tumor* regression. Endogenous expression of *antigen* by DC could offer the potential advantage of allowing prolonged constitutive presentation of endogenously processed epitopes and exploitation of *multiple* restriction elements for the presentation of the same *antigen*. METHODS: DC were prepared from the peripheral blood of HLA A*0201 patients with metastatic melanoma in the presence of IL-4 (1000 IU/mL...

... and fowlpox viruses encoding the hMART-1 gene were constructed and used to infect DC. The efficiency of infection and expression of the MART-1 *antigen* were assessed by immunohistochemistry and intracellular FACS analyses. Cytotoxic lymphocytes (CTL) were generated by the stimulation of CD8+ T cells, with DC expressing the recombinant gene. Reactivity of the CTL was determined at weeks 1 and 2 by the amount of IFN-gamma released. RESULTS: DC were *infected* with recombinant poxviruses and demonstrated specific melanoma *antigen* expression by immunohistochemistry, immunofluorescence, and intracellular FACS analysis. The expression by DC of MART-1 MAA after viral infection was sufficient to generate CD8+ T lymphocytes that recognized naturally processed epitopes on *tumor* cells in 10 of 11 patients. CONCLUSIONS: Human DC are receptive to infection by recombinant poxviruses encoding MAA genes and are capable of efficiently processing and presenting these MAA to cytotoxic T cells. The potential advantage of this approach is the ability to present specific *antigen* independent of the identification of the epitope or the MHC restriction element. This strategy may be useful for the identification of relevant epitopes for a...

...; Vectors--immunology--IM; Granulocyte-Macrophage Colony-Stimulating Factor--immunology--IM; Interferon Type II--immunology--IM; Interleukin-4 --immunology--IM; Poxviridae--genetics--GE; Poxviridae--immunology--IM; *Tumor* Cells, Cultured

?ds

Set	Items	Description
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            ?)) (S) (ARR[redacted] OR MULTIPLE OR POLYEPITOPE?)
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S3          0   S2 AND (DIFFERENTIAL (W) (DISPLAY OR SCREENING OR EXPRESSI-
              ON))
S4         78   S2 AND (TUMOR OR CANCER)
S5         36   RD (unique items)
S6          4   S5 AND REVIEW
S7         11   S5 NOT PY>1998
S8      38901   (ARRAY OR MULTIPLE) (S) (ANTIGEN OR ANTIGENS)
S9       1925   S8 (S) ((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (-
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S10       229   S9 (S) (TRANSFECTED OR TRANSFORMED OR INFECTED OR (GENETIC-
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S11        99   S10 AND (TUMOR OR CANCER)
S12         0   S11 AND (DIFFERENTIAL (W) (DISPLAY OR EXPRESSION OR SCREEN
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S13        47   RD S11 (unique items)
S14        11   S13 NOT PY>1998
S15         2   S14 NOT S7
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               4  SCREENIN
           89065  DENDRITIC
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          468466  MODIFIED
           19839  GENETICALLY(W)MODIFIED
          142085  TRANSFECTED
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?s s18 and (vector)
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     S19       1  S18 AND (VECTOR)
?t s19/3,k/all

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GM-CSF induces specific patterns of cytokines and chemokines in the skin:
  Implications for DNA vaccines.
AUTHOR: Perales Miguel-Angel(a); Fantuzzi Giamila; Houghton Alan N(a);
  Dinarello Charles A; Wolchok Jedd D(a)
AUTHOR ADDRESS: (a)Laboratory of Solid Tumor Immunology, Memorial
  Sloan-Kettering Cancer Center, New York, NY**USA
JOURNAL: Blood  96 (11 Part 1):p456a November 16, 2000
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RECORD TYPE: Abstract
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SUMMARY LANGUAGE: English

ABSTRACT: Granulocyte-macrophage colony-stimulating factor (GM-CSF) has the ability to enhance immune responses through a number of mechanisms. It acts directly on *dendritic* *cells* by inducing their proliferation, maturation and migration, thus increasing their function as antigen presenting cells. It also contributes to the expansion and differentiation of B and T lymphocytes. Similar biological effects have been observed with the use of GM-CSF DNA in mouse models for *cancer* and infectious diseases, and its use is currently being investigated in clinical trials in combination with DNA vaccines. DNA vaccination can induce antibodies or CD4...

...murine GM-CSF DNA under the CMV-promoter via gene gun to the skin. Measurement of various cytokine and chemokine levels in the skin reveals *differential* *expression* patterns over time. The early response is characterized by high levels of inflammatory molecules such as IL-1beta, IL-6, TNF-alpha, and the beta...

...response peaks at 48 hours and is maintained beyond 192 hours, when other cytokines have returned to baseline. Significantly lower responses are seen with the *vector* alone or the GM-CSF gene in the anti-sense orientation. In addition, GM-CSF DNA induces measurable levels of GM-CSF protein both in...

...to DNA vaccines. They give an indication of the cells that are being recruited from the circulation and provide models to develop new approaches to *cancer* vaccines, including the use of cytokine genes as molecular adjuvants.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...TNF-alpha {*tumor* necrosis factor-alpha
...
?ds

Set	Items	Description
S1	2837	((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (W) CELL-?)) (S) (ARRAY OR MULTIPLE OR POLYEPITOPE?)
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S3	0	S2 AND (DIFFERENTIAL (W) (DISPLAY OR SCREENING OR EXPRESSION))
S4	78	S2 AND (TUMOR OR CANCER)
S5	36	RD (unique items)
S6	4	S5 AND REVIEW
S7	11	S5 NOT PY>1998
S8	38901	(ARRAY OR MULTIPLE) (S) (ANTIGEN OR ANTIGENS)
S9	1925	S8 (S) ((ANTIGEN (W) PRESENTING (W) CELL?) OR (DENDRITIC (-W) CELL?))
S10	229	S9 (S) (TRANSFECTED OR TRANSFORMED OR INFECTED OR (GENETICALLY (W) MODIFIED))
S11	99	S10 AND (TUMOR OR CANCER)
S12	0	S11 AND (DIFFERENTIAL (W) (DISPLAY OR EXPRESSION OR SCREEN OR SCREENING))
S13	47	RD S11 (unique items)
S14	11	S13 NOT PY>1998
S15	2	S14 NOT S7
S16	206	(DIFFERENTIAL (W) (DISPLAY OR EXPRESSION OR SCREEN OR SCREENIN)) (S) (DENDRITIC (W) (CELL OR CELLS))
S17	0	S16 (S) ((GENETICALLY (W) MODIFIED) OR TRANSFECTED)
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